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JOURNAL  
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Volume 49

September, 1933

No. 1

PROCEEDINGS OF THE THIRTY-SECOND ANNUAL MEETING  
OF THE NORTH CAROLINA ACADEMY OF SCIENCE

DAVIDSON COLLEGE, DAVIDSON, N. C., MAY 5 AND 6, 1933

The thirty-second annual meeting of the North Carolina Academy of Science was held at Davidson College, May 5 and 6, 1933. The meeting was called to order at 9:30 A.M. on May 5, by the president, Dr. J. B. Bullitt. The reading of papers was begun and continued until 12:50 P.M., when the president appointed the following committees:

Auditing: E. T. Browne, W. N. Mebane, B. W. Wells.

Nominating: Z. P. Metcalf, J. P. Givler, W. F. Prouty.

Resolutions: I. V. Shunk, A. D. Shaftesbury, Vera Koehring.

The Academy then took a recess for a luncheon given by the College to the Academy.

The reading of papers was resumed at 2:15 P.M. and continued until 4:15 P.M., when the Academy went into business session.

The minutes of the previous meeting were approved as published in the *Journal of the Elisha Mitchell Scientific Society*.

Reports were called for from the various committees.

The executive committee consisting of J. B. Bullitt, president of the Academy, Earl H. Hall, vice-president, H. R. Totten, secretary and treasurer, W. L. Porter, F. W. Sherwood, and Charles M. Heck reported as follows:

"The executive committee met at Davidson on May 4 and again on May 5 with the following members present: J. B. Bullitt, W. L. Porter, F. W. Sherwood, Charles M. Heck, and H. R. Totten.

"The committee passed favorably upon the request of L. G. Lehman to substitute for the title printed on the program the title 'Occurrence

of the Frog-eye Disease of Soybeans on Stems, Pods, and Seeds.' The committee also passed favorably upon the request of Messrs. Grove and Tucker to exchange the order in which their papers would be called.

"The committee reported as elected to membership since the last annual meeting the following:

Adams, Louise, Professor of Mathematics, Davenport College, Lenoir, N. C.

Allen, Sallie, Graduate Student in Biology, Duke University, Durham, N. C.

Barnhardt, J. J., Vice-president Cannon Mills Co., Concord, N. C.

Boliek, Irene, Graduate Student in Zoology, U. N. C., Chapel Hill, N. C.

Brownlee, Anne M., Assistant Professor of Biology, Meredith College, Raleigh, N. C.

Carpenter, David W., Instructor in Physics, Duke University, Durham, N. C.

Cathey, Nancy, Instructor in Biology, Queens-Chicora College, Charlotte, N. C.

Clevenger, William L., Professor of Dairy Manufacturing, State College, Raleigh, N. C.

Conrad, Mary E., Professor of Biology, Catawba College, Salisbury, N. C.

Crittenden, Charles, Assistant Professor of Biology, W. C. of U. N. C., Greensboro, N. C.

Dendy, J. S., Assistant in Zoology, U. N. C., Chapel Hill, N. C.

Ferguson, Berdie, Professor of Chemistry and Biology, Davenport College, Lenoir, N. C.

Garner, L. L., Assistant Professor of Mathematics, U. N. C., Chapel Hill, N. C.

Gray, Irving E., Assistant Professor of Zoology, Duke University, Durham, N. C.

Greene, Eloise E., Head of Department of Biology, Queens-Chicora College, Charlotte, N. C.

Grove, C. S., Assistant Professor of Chemical Engineering, State College, Raleigh, N. C.

Hanson, Isobel, Graduate Student in Physics, Duke University, Durham, N. C.

Hauser, C. R., Instructor in Chemistry, Duke University, Durham, N. C.

Howell, Thelma, Professor of Biology, W. C. T. C., Cullowhee, N. C.

Kjellisvig, E. N., Graduate Student in Geology, U. N. C., Chapel Hill, N. C.

Klenner, F. R., Graduate Student in Medicine and Zoology, Duke University, Durham, N. C.

Lawrence, Alfred S., Rector of Chapel of the Cross, Chapel Hill, N. C.

Park, H. V., Teaching Fellow in Mathematics, U. N. C., Chapel Hill, N. C.

Rice, Nolan E., Department of Zoology, Duke University, Durham, N. C.

Roberts, John H., Assistant Professor of Mathematics, Duke University, Durham, N. C.

Robertson, Lora Lee, Teacher of Biology, Mitchell College, Statesville, N. C.

Roth, S. G., Department of Mathematics, U. N. C., Chapel Hill, N. C.

Smith, William L., Department of Physics, Catawba College, Salisbury, N. C.

Tiedeman, John O., Assistant Professor of Physics, W. C. of U. N. C., Greensboro, N. C.

Wood, V. E., Professor of Physical Science, Mars Hill College, Mars Hill, N. C.

• Reinstated to membership:

Douglas, J. M., Professor of Physics, Davidson College

Harbison, T. G., Botanist, Highlands, N. C.

Hood, Frazer, Professor of Psychology, Davidson College

Nielsen, Walter M., Professor of Physics, Duke University

Stokes, Ruth W., Department of Mathematics, Duke University

Complimentary membership for the year 1933:

Foster, Norman B., Oteen, N. C.

"The committee also reported the following losses during the year:

Lost by death:

Rev. George W. Lay, Chapel Hill, N. C., died August 12, 1932.

Lost by resignation:

Wilburt C. Davison

G. D. Collins

Thorndike Saville

Mary L. Sherrill

Lost after removal from the state:

Avery, George S.

Beaumont, J. H.

Brown, Frank N.

Perry, J. Whitney

Pleasants, Annie Lewis

Scott, E. R.

Dropped from the roll for the non-payment of dues:

Twenty-one former members.

"The committee accepted the invitation of the University of North Carolina to hold the thirty-third annual meeting at Chapel Hill.

"The executive committee made the following recommendations to the Academy:

1. "That all bills presented in the Treasurer's Report be authorized and paid, and that the report be printed when audited."

2. "That H. B. Arbuckle be appointed a committee of one to select the cup to be given to the winner of the High School Science Essay Prize,

and that he be authorized to draw upon the Treasury for as much as \$25.00 for the cup; and that the Academy send a representative to the commencement of the Greensboro High School.

3. "That the Academy elect to life membership Dr. J. L. Lake, Professor Emeritus of Physics in Wake Forest College. Dr. Lake joined the Academy in 1902, was its vice-president in 1907 and its president in 1922. Dr. Lake was retired from active teaching at the commencement of Wake Forest College, 1932.

4. "That, after this year's meeting, papers presented before the General Section shall be limited to ten minutes each.

5. "That on next year's program the first hour after luncheon on Friday be set aside for the presentation of invited papers by non-members of the Academy.

6. "That twenty to twenty-five minutes be set aside in the Friday evening program for a review of the year's notable scientific achievements in North Carolina."

The Academy considered the recommendations of the executive committee by section and made the following disposition of them:

Sections 1, 2, and 3 were adopted. For section 4 the Academy refused to limit papers presented before the General Section to ten minutes, and voted to retain the fifteen minute limit. The Academy debated section 5 as to inviting non-member speakers but the sentiment was strongly against it and the recommendation was laid upon the table. A motion was then made that the executive committee be authorized to invite a distinguished speaker or speakers to contribute to the evening program, but the motion was lost. Resolution 6 was also lost.

The Treasurer's report was as follows:

#### *Financial Statement*

1932-1933

Receipts		Expenditures	
Balance on hand, May 6, 1932..	\$530.61	Stationery and postage.....	\$47.67
Dues for		Printing, mimeographing,	
1931.....	2.00	and addressographing.....	41.80
1932.....	142.00	Telegrams and telephoning...	1.94
1933.....	206.00	Journal of the E. M. S. S.	
Initiation fees:		(partial payment).....	250.00
At 1932 meeting.....	14.00	Clerical assistance.....	70.00
Since 1932 meeting.....	48.00	H. S. Essay Prize.....	23.00
Interest on Savings.....	20.14	Dues refund to Sec'y.....	2.00
Allotment from A. A. A. S...	42.50	Sec'y-Treas. Commiss.....	39.20

Receipts		Expenditures	
Chemists' programs.....	5.00	Expenses of Academy representative to inauguration of President Boylan.....	5.00
Replacement of checks.....	5.98	Suspended checks (Banks closed).....	14.50
	<hr/>	Refunding replaced checks later collected.....	3.98
	\$1016.23	Tax.....	.58
			<hr/>
		To balance.....	\$499.67
			516.56
			<hr/>
			\$1016.23
		Outstanding obligations:	
		Balance due the Journal of the E. M. S. S.....	50 00
	Comparison		
	1932	1933	
Savings account.....	\$496.89	\$496.89	
Checking account.....	21.72	13.67	
Cash on hand.....	12.00	6.00	
	<hr/>	<hr/>	
	\$530.61	\$516.56	
Outstanding obligation.....	0	50.00	
	<hr/>	<hr/>	
	\$530.61	\$466.56	
To balance (net loss).....		64.05	
		<hr/>	
		\$530.61	

The above report was made as of May 3, 1933.

Submitted by

H. R. Totten, *Secretary-Treasurer*.

Audited by

W. N. Mebane, Jr.

Edward T. Browne.

B. W. Wells.

Date May 5, 1933.

The auditing committee reported that they had examined the accounts of the treasurer for the period May 6, 1932, to May 5, 1933, and found said accounts in good order.

The reports of the treasurer and the auditing committee were accepted.

The committee on high school science consisting of Bert Cunningham, chairman; H. B. Arbuckle, Lena Bullard, C. M. Heck, C. E. Preston, and R. N. Wilson, reported as follows:



"The committee recommends the continuation of the high school essay prize.

"The committee recommends that it be permitted to consider the possibility of awarding annually two prizes in the place of one; one to be given in the fields of physics and chemistry, the other in the fields of biology (zoology, botany, physiology) and physical geography, with power to act in case its expenditure does not exceed that allotted at the present time.

"The State Department coöperated in sending out the notice of the prize this year, and much of the splendid response may be attributed to their interest in this project. The 'notice' contained much more than the usual announcement. The committee suggests that the Academy express its appreciation of this coöperative spirit on the part of the State Department.

"As usual the various section meetings of the N. C. E. A. were attended by members of the committee who entered into the discussions, sometimes making the principal address. Several members attended the N. C. E. A. state meeting in Raleigh.

"The chairman of your committee has had calls for lecturers, and has recommended certain members of the Academy to the various schools. It is desirable that more members who are willing to do this type of service for the expenses involved should state their willingness to the chairman of the committee.

"The committee elected the following judges for the essay prize:

"E. H. Hall, J. N. Couch, and T. E. Powell, who announce the following decision:

Number of Contestants: 46. (This includes only those papers submitted to the judges. Each school was allowed to submit three of its most worthy papers.)

Number of Schools competing: 26.

Winner: Lane Barksdale of Greensboro Senior High School

Title: Orchid Hunting in Guilford County.

"The committee recommends that Dr. H. B. Arbuckle be authorized to purchase the cup, and that the secretary be authorized to appoint a delegate to present it."

The report of the committee on high school science was accepted and its recommendations were adopted.

The committee to coöperate with representatives of the North Carolina College Conference to study the question of standardization of

college science courses and to represent the Academy at the North Carolina College Conference, consisting of Bert Cunningham, chairman, P. M. Ginnings, Karl H. Fussler, J. B. Bullitt, president of the Academy, and H. R. Totten, secretary of the Academy (the last two *ex-officio* members), gave the following report of progress made.

"The committee met with Dr. J. H. Hillman, chairman of the committee on standardization of science teaching in colleges and universities of the North Carolina College Conference in Durham on June 6, 1932.

"Dr. Hillman reviewed the results of a survey that he had recently conducted, listing the various courses in science now taught in the colleges and universities of the state. He then raised the question as to how far standardization should be attempted.

"After a rather full discussion the attitude of the committee was as follows:

1. a. "That for introductory courses standardized names, such as, General Biology, or Introductory Biology; General Botany, or Introductory Botany; General Zoology, or Introductory Zoology; General Chemistry, or Introductory Chemistry; General Physics, or Introductory Physics, would be desirable.

- b. "That beyond the elementary courses it would be difficult to standardize names or numbers of courses. Sequences and special courses are extremely varied.

2. "That the standardization of credit hours would be impractical as some schools are on a quarter basis, some on a semester basis; some believe in short courses, others in courses more extensive. Standardization in this line would of necessity be a compromise forcing a change of organization of all present existing elementary courses.

3. "Content should be left largely to the instructor who should be sufficiently trained to take advantage of the local natural resources and should mould his course to most satisfactorily meet the needs of his students. The *importance of the teacher* was stressed.

4. "That the supplies and equipment should be sufficient for the course in the region in which it is taught; that it is perhaps advisable to recommend a minimum monetary value for equipment and supplies; but that the *usability* of the equipment should be stressed.

5. "The committee was emphatically opposed to any general syllabus scheme which would dictate the direction of the course. The content should be left to the individual department and the credit to the individual institution. The emphasis should not be put upon the following

of a syllabus, but upon work done by the student. This is a matter resting largely upon the instructor. If he is competent, he will restrict his teaching to such courses as can be given satisfactorily.

"Bert Cunningham, J. B. Bullitt, and H. R. Totten also attended the North Carolina College Conference held in Durham in the fall and heard the report of the Conference Committee on the same subject. No significant action was taken at the College Conference other than to continue the committee, with the understanding that they were to report again the following fall.

The report of the committee was accepted and the committee continued.

The legislative committee made up of Dr. Z. P. Metcalf, chairman, W. L. Poteat, and C. S. Brimley, reported that the activities of the last legislature had not been of such a nature as to call this committee into action. The report of the committee was accepted and the committee was continued.

The special committee in honor of Rev. George W. Lay, consisting of J. S. Holmes, chairman, H. V. Wilson, H. H. Brimley reported as follows:

REVEREND GEORGE WILLIAM LAY, D.C.L.

"George William Lay who departed this life August 12, 1932, was born in Huntsville, Alabama, in 1860, the son of the Right Reverend Henry C. Lay, Missionary Bishop of the then Episcopal District of the Southwest. He was educated at St. Paul's School, Concord, New Hampshire, and at Yale where he was graduated in 1882. Following three years at the General Theological Seminary, New York, he served churches at Erie, Pennsylvania, and Newburgh, New York, and then went back as Master to his own school at Concord. After nineteen years successful work there as a teacher of boys, he was called to the rectorship of St. Mary's School, in Raleigh, N. C., in 1907. For eleven years he was in charge of this important school and under his administration the school greatly increased in size and influence. On resigning as Rector of St. Mary's, Doctor Lay took up parochial work again, serving first at Springfield, Massachusetts, and then at Beaufort, North Carolina. In 1928 he retired from active ministerial work and went to live in Chapel Hill. Even after this he took temporary charge of the Good Shepherd parish at Raleigh for a period of eight months with great profit to the congregation and his own real satisfaction.

"He married in 1894 Anna Booth Balch, daughter of Rear Admiral

George Beall Balch of the United States Navy, who, with her five daughters and two sons, now survives him.

"Teaching and preaching constituted Doctor Lay's life work, and for both of these he was especially fitted by reason of his thoroughness, accuracy, and fairness.

"But his hobby and avocation was science. Science with him was a recreation, not his profession; however, he read widely and understood deeply; and without calling himself a scientist, few men were better acquainted with the findings of modern physical science than he. He was imbued with the true scientific spirit of thoroughness, accuracy, and humility. His later contributions to the programs of the North Carolina Academy of Science were along the line of philology and, in tracing the derivation and developed meaning of words, he did not hesitate to criticise scientific terms in common use; his papers were always an occasion for relaxation and enjoyment.

"Doctor Lay loved trees. Not so much from the point of view of the forester or botanist, but as seen by the outdoor man to whom a tree is an individual entity, worthy of admiration for its beauty, often worthy of respect for its age and always worthy of interest and study for its individual characteristics.

"He was profoundly interested in the theories and the facts pertaining to evolution. The last extended conversation one member of this Committee had with Doctor Lay was just after the publication of the "The New Evolution," by Austin A. Clark, about two years ago, and it was mainly confined to the newest theories on the subject advanced by the writer of the book. But as neither was at that time very familiar with the work, so neither was in a position to be a very severe critic or an ardent supporter of Dr. Clark's theories. The interest of Doctor Lay in the whole subject was, however, keen and intelligent.

"Doctor Lay had been a faithful and stimulating member of the Academy for many years and in his death the Academy has lost a valued member and each member a good friend. In his life as a clergyman and a citizen, he was recognized as a lover of the truth and one who lived and preached righteousness.

H. V. WILSON,  
H. H. BRIMLEY,  
J. S. HOLMES, *Chairman.*

The report was accepted and the secretary was instructed to place the report in the minutes.

The general resolutions committee reported as follows: 1. "In the

death of Dr. Clarence A. Shore of the State Laboratory of Hygiene at Raleigh we recognize the loss of one of the most progressive and energetic workers in the state in regard to preventive medicine. Dr. Shore's reputation among the medical men of the state and nation has been of the highest. Therefore, be it resolved that the North Carolina Academy of Science acknowledge the irreparable loss which it and the state of North Carolina have suffered in his passing.

2. "The North Carolina Academy of Science will miss the guidance of Rev. George W. Lay of Chapel Hill who was always present at our annual meetings. He was an enthusiastic member and preeminently fostered high standards in scientific expression. He was found ever strong for the best interests of our Academy. Therefore, be it resolved that in his death we have lost an enthusiastic and useful member and one whose place cannot be filled by another.

3. "The Academy desires to extend its sympathy to Dr. H. B. Arbuckle in the recent loss of his brother.

4. "And be it further resolved that a copy of these resolutions be spread on the minutes of the Academy and that the secretary be instructed to send copies of suitable portions of these resolutions to the bereaved families.

5. "We wish to express our sincere appreciation to the trustees, administration, and faculty of Davidson College for the excellent and unbounded hospitality extended to the Academy on this the second visit to their campus. The pleasant memories of our visit will remain with us for many years to come.

6. "Resolved that the North Carolina Academy of Science place itself on record as vigorously opposed to the limitation of funds to be raised locally for the support and extension of school terms.

I. V. SHUNK,  
VERA KOEHRING,  
A. D. SHAFTESBURY.

The above resolutions were adopted.

The nominating committee submitted the following nominations:

*President*—B. W. Wells, State College.

*Vice-president*—Helen Barton, Woman's College.

*Secretary-Treasurer* (for three years)—H. L. Blomquist, Duke University.

*New Member of the Executive Committee* (for three years)—E. T. Browne, University of North Carolina.

*Representative to the American Association* (for two years)—C. F. Korstian, Duke University.

The secretary was instructed to cast the ballot of the Academy for the nominees.

Under the unfinished business a motion was made and carried that the greetings of the Academy be sent to Professor Norman B. Foster, now at Oteen.

Under new business H. B. Arbuckle made the suggestion that a vote be made to ascertain the wishes of the Academy as to whether the Academy at this time desired further division of sections such as botanical and zoological sections for the Saturday morning sessions. A motion to that effect was made but was not seconded.

Z. P. Metcalf made a motion that the Academy go on record as expressing its appreciation to H. R. Totten for his years of service. The motion was carried.

J. P. Givler passed to the Academy a suggestion from Earl H. Hall that books be substituted for cups as the high school science essay prize. A motion was made and carried that the high school science committee be so instructed.

The president appointed to the high school science committee for the following year: Bert Cunningham (chairman), Duke University; H. B. Arbuckle, Davidson College; Lena Bullard, Greensboro High School; C. M. Heck, State College; C. E. Preston, University of North Carolina; R. N. Wilson, Duke University.

The business meeting then adjourned.

The Academy reconvened at 8:30 P.M. with W. L. Porter from the executive committee presiding in the absence of Vice-president Earl H. Hall. J. M. McConnell, Dean of the Davidson College Faculty, welcomed the Academy to Davidson. W. L. Porter presented President J. B. Bullitt who then delivered his presidential address on "Early Men, Some Comparisons, not Odious" (Lantern).

The Academy convened in sections Saturday morning with President Bullitt presiding over the general section; F. W. Sherwood, over the chemists, R. W. Bost, as secretary of the chemistry section; J. M. Thomas over the mathematicians, Helen Barton as secretary; H. E. Fulcher over the physicists, C. N. Warfield as secretary.

The following group officers were elected by the group concerned for the year 1934:

*North Carolina Section of the American Chemical Society*—Chairman,

H. D. Crockford, University of North Carolina; Vice-chairman, J. E. Saylor, Duke University; Secretary-Treasurer, R. W. Bost, University of North Carolina; Councilor, N. Isbell, Wake Forest College; Executive committee, the officers and F. W. Sherwood, N. C. Agricultural Experiment Station (three years), L. A. Bigelow, Duke University (two years), and A. S. Wheeler, University of North Carolina (one year).

*Mathematics*—Chairman, E. L. Mackie, University of North Carolina; Secretary, E. R. C. Miles, Duke University.

*Physics*—Chairman E. K. Plyler, University of North Carolina; Secretary, C. N. Warfield, Woman's College of the University of North Carolina.

The following papers were presented. Those marked with \* appear in full in this issue. Those marked x are abstracted in this issue. For those unmarked no abstract was received. Those marked † were presented by title only.

#### GENERAL SECTION

*Effects of Increased Atmospheric Pressure on Developing Eggs of the Hen* (Lantern). BERT CUNNINGHAM, Duke.

\**Occam's Razor and Mendel's Peas*. J. P. GIVLER, W. C. of U. N. C.

x*Notes on the "Depression Plant."* O. J. THIES, JR., Davidson.

*Spirochetes in the Cat with Special Reference to those of the Alimentary Tract* (Lantern). ELOISE E. GREENE, Queens-Chicora.

†*Studies of Different Nitrogenous Fertilizers with Mature Peach Trees*. C. F. WILLIAMS, State.

x*A Review of the Fleas of North Carolina with Special Reference to Sex Ratios* (Lantern). A. D. SHAFTESBURY, W. C. of U. N. C.

\**A Photographic Method of Collecting References*. T. B. MITCHELL, State.

†*The Carolina Lake District* (Lantern). COLLIER COBB, U. N. C.

x*Ultrapaque Microscope Equipment as an Aid in Stomatal Studies* (Lantern). I. D. JONES, State.

*An Interesting Epidermal Cell Wall* (Lantern). D. B. ANDERSON, State.

x*Narcotic Phenomena in Asterias* (Lantern). VERA KOEHRING, Beaufort Lab.

x*Metallized and Irradiated Milk in the Treatment of Nutritional Anemia* (Lantern). J. L. MCGHEE and BERTIE FERGUSON, Davenport.

x*Chalcid Conjury* (Lantern). B. B. FULTON, State.

- †*Some Disease Symptoms on Plants Resulting from Cold Injury* (Lantern). R. F. POOLE, State.
- x*Cell Division in Oedogonium Kurzii* (Lantern). L. A. WHITFORD, State.
- x*Extra-nuclear Activities during Reduction Division in Anthoceros carolinianus* (Opaque Lantern). H. L. BLUMQUIST, Duke.
- Development of the Peristome in a Double Peristomate Moss, Aulacomnium heterostichum* (Lantern). LORA LEE ROBERTSON, Mitchell.
- Studies in the Sexuality of Dictyuchus* (Lantern). J. N. COUCH and MARY LINDA VARDELL, U. N. C.
- Observations on Downy Mildew of Tobacco*. FREDERICK A. WOLF, Duke.
- Occurrence of the Frog-eye Disease of Soybeans on Stems, Pods, and Seeds*. L. G. LEHMAN, State.
- \**Removal of Manganese from Water Supplies*. E. E. RANDOLPH, State.
- \**Fatal Poisoning with Sodium Nitrite*. H. B. ARBUCKLE and O. J. THIES, JR., Davidson.
- A Detailed Dot Map of the Distribution of Population in North Carolina*. CHARLES CRITTENDEN, W. C. of U. N. C.
- A Coast Transect* (Lantern). B. W. WELLS, State.
- x*Some Magnetometer Observations in the Coastal Plain Area of South Carolina*. GERALD R. MACCARTHY, W. F. PROUTY, and J. A. ALEXANDER, U. N. C.
- x*Some Geophysical Experiments*. J. A. ALEXANDER, U. N. C.
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- Rhododendrons and Azaleas of the Southeastern United States*. T. G. HARBISON, Highlands.
- Seed Development in Spigelia marylandica*. MARY LINDA VARDELL, U. N. C.



x*Degeneration Phenomena in Sponge Larvae* (Lantern). H. V. WILSON and IRENE BOLIEK, U. N. C.

†*Cenotes*. A. S. PEARSE, Duke.

*Vincent's Angina: Etiology and Treatment with Hexylresorcinol Solution S.T. 37*. B. J. BAROODY, Duke.

\**Modern Zoogeography* (Lantern). Z. P. METCALF, State.

†*The Histo-physiogenesis of the Testis of the Bull* (Lantern). C. W. HOOKER, Duke.

*Temperature Effects on Developmental Rates of Turtle Eggs* (Lantern). BERT CUNNINGHAM, Duke.

*Marble Deposits of North Carolina*. J. L. STUCKEY and JAMES FONTAINE, State.

x*Foundation Problems in the Morgan Creek Dam of the University of North Carolina*. W. F. PROUTY, U. N. C.

†*Sand Grains and their Shapes*. GERALD R. MACCARTHY, U. N. C.

x*Notes on a New Eurypterid from the Moscow Shales of New York*. E. N. KJELLESVIG, U. N. C.

x*Industrial Quality of Public Water Supplies of the Coastal Plain*. E. E. RANDOLPH, C. S. GROVE, and J. C. MCNAIR, State.

#### MATHEMATICS SECTION

x*Symbolic Cubic Forms in Six Variables*. RUTH W. STOKES, Duke.

x*Analytic Criteria for Geometric Conditions*. H. V. PARK, U. N. C.

x*On the Forms of Equations of Curves*. S. G. ROTH, U. N. C.

x*The Classification of Collineations in the Plane*. E. T. BROWNE, U. N. C.

x*Integral Equations with Solutions of Limited Variation*. F. G. DRESSEL, Duke.

x*Certain Conics Associated with Non-singular Correlations in the Plane*. L. L. GARNER, U. N. C.

x*Open Subsets of Non-compact Continua*. JOHN H. ROBERTS, Duke.

†*The Expanding Universe and the Age of the Earth*. A. HENDERSON, U. N. C.

†*Reduction of a Pfaffian to Canonical Form*. J. M. THOMAS, Duke.

#### PHYSICS SECTION

x*The Elongation of a Rubber Band as a Function of the Number of Applications of an Intermittent Stretching Force*. M. L. BRAUN, Catawba.  
*Comparison Between Theoretical and Experimental Coefficients of Viscosity of Helium*. W. M. NIELSEN, Duke.

*The Effect of Electron Temperature on the Spectrum of Mercury Vapor.* M. M. MANN, Duke.

*The Effect of Temperature on the Rate of Diffusion of Metastable Mercury Atoms.* D. W. CARPENTER, Duke.

*The Saturation Magnetization of Cobalt.* F. W. CONSTANT, Duke.

*Diamagnetism and Paramagnetism in Intense Fields.* F. W. CONSTANT and D. H. MOORE, Duke.

*The Effect of Temperature on the Raman Spectrum of Carbon Dioxide Gas.* I. HANSON, Duke.

*Radio Fading.* CHARLES HECK, State.

#### EXHIBITS

*Fleas.* ARCHIE D. SHAFTESBURY, W. C. of U. N. C.

*A Detailed Dot Map of the Distribution of Population in North Carolina.*

CHARLES CRITTENDEN, W. C. of U. N. C.

*A Photographic Method of Collecting References.* T. B. MITCHELL, State

*Azaleas of the Southeastern United States.* T. G. HARBISON, Highlands.

*Commercial Exhibits:*

Phipps and Bird, Richmond, Va.

Ethyl Gasoline Corporation, New York, N. Y.

#### NORTH CAROLINA SECTION OF THE AMERICAN CHEMICAL SOCIETY

*The Salting Out of Non-electrolytes by Electrolytes (Lantern).* J. H. SAYLOR, P. M. GROSS, and A. T. WHITENFISH, Duke.

*The Determination of Malic Acid.* E. W. MCCHESENEY, U. N. C.

*Ternary Systems: Water, Pyridine, and Salts at 25 degrees.* P. M. GINNINGS, BAILEY WEBB, and EMI HINOHARA, G. C.

*Vapor Pressures of Some Organic Ethers and Thioethers.* H. D. CROCKFORD and R. H. MUNCH, U. N. C.

*Elementary Fluorine in Organic Chemistry.* LUCIUS A. BIGELOW, Duke.

*Chemical Composition of Melia Azedarach.* R. W. BOST, U. N. C.

The following abstracts have been received:

*Notes on the "Depression Plant."* O. J. THIES, JR.

The depression flower, a chemical growth consisting largely of salt on coal, brick, twigs, etc., has attracted widespread attention. Many explanations have been offered for the growth, but heretofore all have omitted the bluing called for in the formulas. (Common formulas call for salt, bluing, water, and an alkaline substance.)

The growth will not occur on brick, coal, etc., when pure salt is used alone, nor when blue ink, liquid bluing, ferri ferrocyanide, saponin, sodium bicarbonate, sodium carbonate, potassium ferricyanide, sodium sulphite, copper nitrate, copper nitrate with copper ferrocyanide, mercurochrome, potassium chromate, ammonium hydroxide, lime, magnesium carbonate, sodium dichromate, and ferrous sulphate, respectively, are used with the salt. Potassium dichromate, potassium cobalticyanide, and sodium cobaltocyanide, respectively, with salt produced a suggestion of growth, but nothing to be compared with that of the depression plant.

Growth was produced when bluing was mixed with the salt in the presence of some alkali, and in almost every case (except in the presence of deliquescent substances, strong acids, and strong alkalis) when salt was present with potassium ferrocyanide, sodium ferrocyanide, ammonium ferrocyanide, or any other substance supplying as much as 0.02% ferrocyanide ion. One tenth of this amount is insufficient to cause the growth of the plant. Best results are obtained with about 0.1% potassium ferrocyanide. Too much ferrocyanide interferes with the growth.

The success of the formulas using bluing is to be attributed to the fact that added alkali, soda or ammonia, or alkali in free-flowing table salt used, reacted with the ferri ferrocyanide of the bluing to produce a soluble ferrocyanide.

Only two substances, potassium chloride and potassium bromide, were found which could be substituted for salt. These behaved much as salt did, and potassium bromide even showed some tendency to grow in the absence of a ferrocyanide. The following produced no growth with or without ferrocyanide: Deliquescent substances, ammonium chloride, sodium iodide, sodium bromide, potassium iodide, sodium fluoride, ammonium nitrate, sodium nitrate, sodium sulphite, borax, trisodium phosphate, sugar, sodium carbonate, and magnesium sulphate.

The growth of the "plant" is dependent on the evaporation of water, leaving behind particles of salt, which are mixed with a small amount of ferrocyanide. The salt particles do not appear typically crystalline, and frequently the growth is tubular, like stalactites.

Potassium ferrocyanide is abnormal in a number of its properties. One of these abnormalities is the fact that its beta form will grow into a depression plant when treated with water and in the absence of salt, whereas the alpha form will not grow into a depression flower. However, either the alpha or beta form will cause salt to grow.

Explanation of the growth of the depression flower was sought in surface tension determinations of solutions of salt and ferrocyanides. Results were so erratic, however, that no conclusions could be drawn. Osmosis and capillarity are both probably intimately connected with this phenomenon.

The results of our experiments lead to the conclusion that the ferrocyanide ion is unique and specific in causing sodium chloride to grow into depression flowers.

*A Review of the Fleas of North Carolina with Special Reference to Sex Ratios.* A. D. SHAFTESBURY.

In an examination of over two thousand specimens of fleas (Order Siphonaptera) from common animals in 38 counties of North Carolina, during the past two years, ten species have been identified, as follows: cat flea (*Ctenocephalides felis*); dog flea (*C. canis*); human flea (*Pulex irritans*); tropical sticktight flea (*Echidnophaga gallinacea*); rabbit flea (*Odontopsyllus multispinosus*); mole flea (*Ctenophthalmus pseudagyrtes*); squirrel flea (*Myxopsylla wickhami*); rabbit flea (*Spilopsyllus simplex*); rat flea (*Ceratophyllus fasciatus*); and the plague flea of rats (*Xenopsylla cheopis*). *Ceratophyllus fasciatus* has apparently not been previously recorded from this state. The data indicate that *Pulex irritans*, *Ctenocephalides canis*, *C. felis*, *Myxopsylla wickhami*, and *Spilopsyllus simplex* are to be expected wherever suitable hosts are found in the state. *Echidnophaga gallinacea* appears to be restricted almost exclusively to the coastal plain region. With all species collected in any considerable numbers, the females predominated, the ratios ranging from 61½% females in *Pulex irritans*, to 86% females in the case of *Echidnophaga gallinacea*. The reason for this apparent differential sex ratio is not evident.

*Utopaque Microscope Equipment as an Aid in Stomatal Studies.* IVAN D. JONES.

Utopaque microscope equipment, which is essentially a modified form of a vertical illuminator, has been used in a study of the stomatal movements of leaves of the peach. With this equipment the guard cells appeared as brightly outlined contours. If the stoma was closed a bright line was seen between the elliptical contour of the guard cells. If the stoma was open a dark area was seen between the guard cells. This dark area varied in shape from a narrow line when the opening was small to nearly circular when the opening was large.

The results obtained by this method compared favorably with those obtained by the "dry-mount" method or the alcohol fixation method. It is believed that the advantages of this method of stomatal observation are that it affords an opportunity to observe the leaf at any time without serious injury, on the living plant if desired; it permits the ready and accurate observation of a great number of stomata at one time and without delay; it makes possible the observation of practically any portion of the leaf surface that is desired.

*Narcotic Phenomena in Asterias.* VERA KOEHRING.

When the starfish *Asterias* is narcotized with ether, chloroform or acid, the stomach is extruded. In the early stages of narcosis the star can be turned oral side up and will remain in this position. It can then be noted that the stomach is very gradually protruded from the mouth opening as narcosis becomes complete. The stomach tissue is fully extruded as it appears. When the stomach is fully extruded the voluminous lobes present an extensive area of decidedly opaque membranes, indicating the coagulation of certain constituents of the protoplasm. Furthermore, a pattern of still more densely coagulated areas is present in a ramification of fine lines throughout the lobes. If the stomach of a narcotized star is compared with the stomach of a normal star which is lifted off of food, great contrast is noted, as the membranes of the normal stomach are transparent. Macroscopically, the normal stomach membranes may be compared to cellophane paper, whereas the narcotized membranes give the appearance of waxed paper. When the narcotized animal is rinsed in fresh sea water, recovery begins and the stomach lobes are slowly withdrawn. Usually before the membranes are entirely withdrawn it is noted that coagulation is being reversed and that the stomach tissue is becoming more and more transparent. The complete test of reversibility is to present the recovered starfish with food within an hour or so of the experiment. The extruded stomach lobes of this feeding animal are found to be normally transparent. This type of material and experiment definitely settles the question of whether or not a distinct and decided coagulation of protoplasmic constituents may occur as a part of the narcotic process, such coagulation being entirely reversible.

*Metallized and Irradiated Milk in the Treatment of Nutritional Anemia.*

J. L. MCGHEE and BERTIE FERGUSON.

Milk, to which copper, iron, and other metals have been added in the

metallic form, was fed to anemic rats. In one week, there was a decided rise in hemoglobin, followed by complete recovery within four to six weeks. This method of treatment has been applied with good results to over one hundred and fifty people, and in many cases, has produced a rapid return to normal. It is thought by the originator of the method that this offers a more satisfactory method of treatment than liver therapy, which is often unpalatable, or use of metallic salts, which produce ill effects due to their acidic hydrolysis.

Irradiation of milk produced no beneficial results in anemia treatment, but was followed by an increased leucocyte count.

*Chalcid Conjury.* B. B. FULTON.

While studying the life history of a Chalcid (*Habrocytus cerealellae*) parasitic on the Angumois grain moth (*Sitotroga cerealella*) certain habits associated with oviposition were observed, which seem at first like conjuring tricks. (1) The ovipositor shaft, which is entirely a chitinous structure with no muscle attachments except at the base, is able to bend near the middle. (2) The elongated egg has a diameter 14 times the diameter of the space in the ovipositor shaft, through which it passes without causing any noticeable expansion. (3) The parasite is able to feed on the blood of the host larva through a tube built up by a secretion from the ovipositor.

The complete paper is to appear in Annals of Entomological Society of America, under the title "Notes on *Habrocytus cerealellae*, parasite of the Angumois grain moth."

*Cell Division in Oedogonium kurzii.* L. A. WHITFORD.

In cell division *Oedogonium kurzii* does not follow the commonly accepted theory but rather that of Kraskovits. After nuclear division is complete a new layer of cell wall material is laid down completely enclosing the protoplast. This layer bulges out near the upper end of the cell to form the familiar ring. The old cell wall ruptures circularly under the ring. The ring stretches out greatly increasing the length of the cell. The cross wall is laid down forming two cells. The lower of these is thus enclosed, except at the top, by two layers while the upper is enclosed only by the single layer formed from the stretched ring.

"Sheath cells" as well as "cap cells" may divide and the walls of the former become progressively thicker at each division by the addition of the new inside layer.

The formation and behavior of the cross wall is not clearly understood.

*Extra-nuclear activities during spore formation in Anthoceros carolinianus*  
Michx. H. L. BLUMQUIST.

A presentation of a study of the behavior of cytoplasm, chloroplasts, vacuoles, and the cell wall in living material of *Anthoceros carolinianus* from the spore-mother-cell stage to the completed spore. This species is especially favorable for such a study, as the sporophyte is unusually large so that all stages may be observed in one sporophyte. The purpose was to observe these stages in a condition which is free from possible chemical and physical effects brought about by the common micro-technical methods. The principal points brought out are as follows: (1) the nucleus of the spore-mother-cell migrates from a parietal to a central position, accompanied by a division of the one large vacuole into many small ones; (2) the original single chloroplast divides into two which migrate to opposite sides of the nucleus followed by a coalescence of the vacuoles into two which alternate in their position with that of the chloroplasts; (3) the two chloroplasts then divide and the resulting four place themselves in a tetrahedral arrangement followed by a similar behavior of the vacuoles, all of the above taking place before the reduction division; (4) during the above events, the spore-mother-cell wall swells inward, crowding the contents of the cell to the center, giving it an extremely plasmolyzed appearance; (5) during reduction division the cytoplasm becomes more dense and stringy and highly refractive; (6) after reduction division the cell contents separate without any wall formation into four equal parts; (7) the wall of the spore is formed from the outer region of the cytoplasm.

*Some Magnetometer Observations in the Coastal Plain Area of South Carolina.* G. R. MACCARTHY, W. F. PROUTY and J. A. ALEXANDER.

In the course of some investigations of the Carolina coastal plain area a magnetometer traverse was run between Myrtle Beach and Darlington, South Carolina. The instrument used was a Schmidt type Askania vertical field balance. Myrtle Beach was taken as the main base station, and all readings were referred to this station as a zero point. A line drawn from Myrtle Beach through Marion was used as a base for the traverse, this line being approximately at right angles both to the coast and to the general trend of the Appalachian structure and therefore cutting the structural trends of the region nearly at right angles.

Between the towns of Homewood and Cool Springs a large plus anomaly was encountered, which reached a maximum of 1600 gammas above

the base station and about 1200 gammas above the regional value. A short parallel traverse made some 8 miles to the northeast also showed a strong plus anomaly near this point, indicating that the anomaly must be linear in nature and elongated parallel to the strike of the general structure. It probably was due to a basic intrusion in the crystalline basement rocks beneath the coastal plain sediments.

A magnetic low about 13 miles wide, reaching a minimum of 400 gammas below the regional value was also found. The city of Florence is located in the center of this low which undoubtedly represents a concealed Triassic lowland, since Triassic rocks have been found at a depth of 608 feet in a deep well drilled at Florence.<sup>1</sup> The magnetometer shows that this lowland has a width of between 12 and 13 miles at this point, and suggests that other concealed Triassic basins may be located by geophysical means.

When the two major anomalies were disregarded and straight lines fitted to the remaining points by the method of least squares it was found that two such straight lines intersecting about 14 miles northwest of Conway were necessary to secure a close fit. The seaward line has a gradient of about 20 gammas<sup>2</sup> to the mile, the landward line one of only 3.26, indicating that at or near this point the surface of the basement crystallines changes its degree of dip, the dip between this point and Myrtle Beach being several times the dip between Darlington and the point in question. This might be interpreted in either of two ways. That is, as the intersection of two erosional surfaces, perhaps the piedmont plateau and the fall-zone peneplains, or as the western margin of a Mesozoic syncline.

It is planned to continue this work as circumstances permit with a view toward working out more fully the structure of the Atlantic coastal plain.

#### *Some Geophysical Experiments.* J. A. ALEXANDER.

After spending the fall and winter quarters studying variations in the earth's magnetic field and measuring natural electrical currents within the earth, members of the geology department extended their geophysical work into the field of water-finding by means of an electrical current forced into the ground.

Ground water carrying salts in solution is a better conductor of elec-

<sup>1</sup> Bull. 138, U. S. G. S. p. 218.

<sup>2</sup> A gamma is the one one-hundred thousandth of a gauss, which latter is the unit employed in measuring strong magnetic fields.



tricity than is dry rock or soil. By measuring the average resistivity to an electrical current at steadily increasing depths under a given point the water table may be located by the lower values given when it is encountered. Set-ups were made beside wells so that the depth to water might be accurately checked. The results seem to be accurate within two or three feet and in eight of eleven wells tested were accurate within one foot. In some of the wells the tests showed that a more abundant water supply could be had by sinking the well only a few feet.

This method could be of much assistance to any one contemplating sinking wells, especially those for artesian supplies.

*Ancient Fossils on Modern Beach.*      W. F. PROUTY and G. R. MAC-CARTHY.

During the Easter holidays while engaged with Dr. G. R. MacCarthy and Mr. J. A. Alexander in a magnetometer reconnaissance of the coastal plain area of South Carolina, and in the study of the meteoric origin of the so-called bays, a Sunday afternoon visit was made to Myrtle Beach. Our party was very much surprised to find great numbers of Cretaceous fossils on the beach mingled with the modern forms. The most numerous of these Cretaceous fossils is an ancient oyster, *Exogyra costata* Say.

Our party picked up also during the hour's stay on the beach twenty-five specimens of an apparently new variety of a comparatively rare species of Upper Cretaceous Echinoid, *Cassidulus emmonsii* Stephenson. Also two other species of Upper Cretaceous oyster. These Cretaceous forms were found over a length of more than two miles of the beach and from the low tide level up to the base of the sand dunes which marks the storm beach. A larger percentage, however, came from the upper and middle portion of the beach. Most of the specimens gathered show but little wear from transportation or wave rolling, though many of the echinoids which are fully fossilized and turned to calcite, have suffered badly by solution of the upper portions. Some of the *Exogyra* have both valves, while others are filled with a dark colored marl or embedded in a hard, marl-like matrix. Dr. J. G. Douglas has made a hurried examination of this marl and reports the presence of *Gumbelina* sp. and *Robulus rotulatus* (Lamarck), characteristic Cretaceous foraminifera.

On looking up the literature we find that two other people have noted the presence of Cretaceous fossils at Myrtle Beach. Earle Sloan<sup>1</sup> in 1906 speaks of the occurrence of Upper Cretaceous fossils on Myrtle Beach and is of the opinion that they have been washed up by wave action from the continental shelf. Mr. Sloan also speaks of the occur-

<sup>1</sup> Catalogue of Mineral Localities of So. Car., page 443.

rence of these fossils in a marl bed along the low water level of the Waccamaw River in a number of localities.

Dr. L. W. Stephenson,<sup>2</sup> Chief of the Coastal Plain Division of the U. S. Geological Survey, also makes reference to the occurrence of fossils of Upper Cretaceous age on Myrtle Beach and also at Windy Hill, nine or ten miles northeast along the beach from Myrtle Beach. Dr. Stephenson is also of the opinion that these fossils are washed up by storm waves from submarine marl beds exposed to wave action.

The near shore submarine "exposure" of Upper Cretaceous marl beds would explain their present distribution by the storm waves along this beach where the sea is gradually encroaching at present on the land. There is, however, an alternative theory equally as reasonable which I wish to propose. A study of the maps of the region and of aerial photographs makes it apparent that in recent geological time the ocean has occupied a position several miles farther inland than now. The waves must, therefore, have done much work on the known outcrop of Upper Cretaceous marls and might well have left numerous Cretaceous fossils buried in the beach sands as it retreated from this area. Also the Waccamaw River, which at present has much of its course through Upper Cretaceous marls, might have brought, in times past, many of these fossils the short distance from the known outcrop to the Myrtle Beach area, at a time when the river had its entrance into the ocean in the neighborhood of Myrtle Beach or to the northeast of Myrtle Beach. Since many of the fossils were embedded in a hard marl they could be transported a considerable distance without suffering much abrasion. It is apparent from a study of the course of the Waccamaw River that it has been turned from its former direct entrance into the ocean by the growth of beach ridges until it now enters the ocean a number of miles to the southwest of Myrtle Beach. Sharp turns in its course in at least two places suggest position of its former entrance to the sea. The shoreward working waves are now removing the former ocean and river sediments and laying bare the buried Cretaceous fossils.

Dr. Stephenson says in a recent letter:<sup>3</sup> "The Myrtle Beach area is the only place along the Atlantic Coast where I know of the occurrence of Cretaceous fossils loose on the beach."

Other localities, however, have been cited for such occurrences. In March, 1906, Professor Collier Cobb<sup>4</sup> speaks of finding Cretaceous fossils along the ocean side of the Currituck Banks.

<sup>2</sup> Cretaceous Report, N. C. Geol. Survey, 1921, p. 34.

<sup>3</sup> April 29, 1933.

<sup>4</sup> Elisha Mitchell Journal 22: 17. 1906.

*Variations in the Test of Nonion pizarrensis Berry and Nonionella auris (D'Orbigny) from the Miocene of North Carolina.* E. N. KJELLESVIG.

This paper is merely a report of progress as it forms a very small part of a detailed description of Miocene Foraminifera which will shortly be released by the Geology Department of the University of North Carolina. The material from which these foraminifera have been secured is a 480 foot well at Elizabeth City, North Carolina. There are fifty-six samples, many of which were taken at five foot intervals. Over half of these samples have been studied and, so far, they have yielded a remarkably prolific micro-fauna which, fortunately, is in an excellent state of preservation.

The variations of the two species here described are of such an unusual nature that it was thought best to describe them in order that we may have a record of their occurrence. *Nonion pizarrensis* and *Nonionella auris* are the commonest forms present in our well samples. The two forms were very common along the coastal plain during Miocene times. Both are living now on the western coast of South America. The two species are very strange in the many variations and distortions that are present in their tests. Minor variations are extremely common, however the writer has selected twenty-five of the more pronounced distortions and has reported on them.

The strangest of all the forms studied was a very abnormal *Nonion pizarrensis*. This individual, instead of being nearly planispirally coiled and involute was entirely evolute and trochoid in the earlier chambers, also was coiled in two planes each at 90° to another; besides being greatly deformed in the earlier stages.

*A Study of North Carolina Clays in Reclaiming Motor Oils.* C. S. GROVE and R. C. TUCKER.

The reclamation of crankcase drainings is a commercial possibility that is at present attracting the attention of all large users of lubricating oils. There have been many processes suggested for such reclamation, of which, the contact clay method seems the best. The number of clay deposits suitable for this type of re-refining is limited and the cost of the commercial varieties is rather prohibitive for the most economical reclamation.

North Carolina with its varied mineral wealth seemed to be a potential source of this type of clay, often known as Fuller's earth. This investigation covered a survey of sixty-four clays from thirty-one counties covering deposits of clays from the mountains to the coastal plains.

It was found that the North Carolina clays, which offered any possibilities of use, were sedimentary in origin, but that even the best of these in the untreated form were so limited in their bleaching action as to practically preclude any feasible utilization of them.

Several South Carolina clays were studied from the lower cretaceous formations. These showed considerable commercial possibilities. It is planned to continue this study of North Carolina clays in the southeastern counties of Robeson, Scotland, Bladen, and Sampson through which the patuxent formation runs—this is a continuation of the South Carolina cretaceous formations. Further study will be done also on the activation of these clays and the colloidal nature of the impurities present in the crankcase drainings.

*Descriptions of Two Amoebae as They Appear in a Standard Culture Medium.* NOLAN E. RICE.

Two amoebae which appeared to be distinct species are described. One is uniformly granular and forms many pseudopodia while the other is not uniformly granular and has a hyaline rim. They are described again as they appeared in a standard culture medium in which the conditions were identical. The one amoeba lost its hyaline rim so that the two amoebae appeared to be identical. Significant differences in the diameters, areas, and average nuclear and karyosomal diameters are pointed out. The amoebae had almost identical average rates of locomotion, but the distribution curves of their rates of locomotion indicate that the amoebae are different. Differences in neutral red granules and mitochondria of the amoebae are demonstrated. Chromatin and thymonucleic acid are shown to have been present in the cytoplasm of both amoebae. Further observations on the amoebae in old cultures showed that the amoebae assumed the forms they had before culture in the standard medium. When the amoebae were grown on 1.5 per cent agar plates made up from the standard medium, they showed distinct physiological differences. Names will be proposed for the two amoebae at a later date.

*The Relation Between Temperature and Locomotion in a Marine Amoeba, Flabellula mira Schaeffer.* D. L. HOPKINS.

The amoebae used in the experiments were collected at Tortugas, Florida. They were cultured at 32–35°C. in natural sea-water to which a definite amount of wheat was added. They were taken from seven-day cultures and placed in artificial sea-water in a micro-incubator

regulated to the specified temperature. The rate of locomotion was ascertained by making camera lucida sketches of an amoeba at minute intervals, and knowing the magnification, the actual rate could be calculated.

The rate of locomotion is always zero at 0°C. and again at 50°C. Above 0°C., the rate gradually increases until a temperature of 35°C. is reached; more rapidly to an optimum at 37.5°C.; decreases to a minimum between 41° and 43°C.; then increases to a second optimum between 45° and 47°C.; and finally falls abruptly to zero at 50°C.

*Degeneration Phenomena in Sponge Larvae.* H. V. WILSON and IRENE BOLIEK.<sup>1</sup>

*Lissodendoryx carolinensis* Wilson is a common shallow water sponge of the North Carolina coast, falling in the Desmacidonidae. The solid ciliated larvae of such sponges is usually described as made up of distinct cells. We find that in *Lissodendoryx* the bulk of the larva consists of continuous protoplasm studded with nuclei, forming a syncytium with which the ciliated ectoderm cells are continuous at their inner ends. The larvae tend to fuse. The composite masses so formed develop like the single larvae, and both singles and composites were used. In metamorphosis the ectoderm as such disappears (details reserved), the ectodermal nuclei moving into the interior. It seems that some of them are always abnormal and are digested or thrown out. Many persist however in normal individuals and no doubt, as in other sponges of this kind, form the centers around which cell bodies differentiate to form the collar cells. The syncytium gradually breaks up into cells which for some time are very imperfectly marked out.

In sponges which are delayed in metamorphosis, some of them infested with bacteria, the bulk of the immigrated ectodermal nuclei become abnormal and the syncytium early breaks up in large part into cells and some multinucleate masses. Many of the cells and masses include degenerate ectodermal nuclei, some of them include bacteria, and still others contain degenerate cells of their own general kind lying in vacuoles. The cells into which the syncytium breaks up are as a class spheroidal with sharp boundaries, i.e. with definite ectoplasmic membranes. A good many are themselves in process of degeneration, as is shown by their pycnotic nuclei. The surface layer of the sponge may be broken down, in which case the free cells wander or fall out.

<sup>1</sup> This work was carried on in part at the Beaufort (N. C.) Marine Laboratory of the U. S. Bureau of Fisheries. We are indebted to the Commissioner for this privilege and to the Director and staff for courtesies and aid.

Sometimes a larva before attachment undergoes a change, which we speak of as "oedema." The interior becomes very fluid and the syncytium breaks up into well separated spheroidal cells with sharp boundaries. In many of them the nuclei are pycnotic. The ectoderm, still at the surface, is also considerably disorganized (details reserved). The tendency of the larva, when sick from one cause or another, to break up before or during metamorphosis into free cells is shown in still another way. At the spicular end of a larva, just entering on metamorphosis, out of the syncytium there may form a few spheroidal cells, which lie in vacuoles and show degenerating nuclei. These cells are discharged and may be found adhering to the intact surface layer of the larva or at some little distance from it.

The tendency of the larval syncytium to break up under adverse conditions into free rounded cells, more often with distinct ectoplasmic membranes, may provisionally be looked on as an adaptive process, as an action-response of the protoplasm that is protective.

*Foundation Problems in the Morgan Creek Dam of the University of North Carolina.* W. F. PROUTY.

The foundation of the recently constructed dam across Morgan Creek shows in an excellent way the type of unsoundness to be expected in the area of the so-called "Slate Belt" with its acid intrusives.

The Morgan Creek Dam has a length of 760' including the three sections. The main section of the dam, built across the main valley, is of the gravity type concrete, 385' long and has a maximum height of about 48'. The earth fill and concrete core part of the dam is 95' long. It is built across a low knoll which separates the main valley from the small valley to the north. The earth fill portion of the dam is 280' long and is built across the shallow valley to the north of the main valley.

The rock foundation is of two general types: The main valley and most of the knoll to the north is underlain by rocks of slaty or schistose character, the so-called Purefoy Slates which are metamorphosed, water-laid, volcanic ash deposits. The planes of slaty cleavage are close together and nearly vertical. They have a strike direction nearly parallel with the stream and practically at right angles to the length of the dam. On the north side of the knoll, and underlying the earth-fill portion of the dam, the rock is a coarse textured granite. The contact between the two types of rock is nearly parallel with the strike of the slates and with the valley.

The foundation problem was largely confined to the area of the gravity type dam. In this portion Morgan Creek had a meander flood plain

with an alluvial deposit from 7'-12' deep. Beneath this flood plain deposit the rocks were found to be fairly free from weathering. On the knoll, at the north end of the main dam, a two foot soil cover graded down into solid rock at a depth of about 12'. Between the soil cover and the unweathered rock was a gradual transition from very soft rock to sound rock. At the south end of the dam the valley slope rises to an elevation of over a hundred feet above the flood plain, while the knoll on the north end is only about 35' higher than the flood plain. At the south end of the dam the rock as a whole is less deeply weathered than in the flatter land at the north end, but along the shear zones weathering has taken place to a greater depth on account of the greater elevation of the surface above the flood plain water table. At the extreme south end of the dam weathering along these shear zones has gone down to a depth of about 18'. Much of the rock between these shear zones was fairly sound and some of it perfectly sound.

The types of unsoundness occurring in the foundations are:

First: Joints, of which there are two very distinct types: (a) the *strike* joints for the most part with planes steeply inclined toward the west and a few more gently inclined toward the east, and (b) the *dip* joints whose trend is nearly that of the dam and whose inclination is generally toward the west, or in this case, upstream.

Second: Faults. These can be classified into (a) single or simple faults and (b) shear zone or multiple faults.

The shear zones are largely parallel with the schistosity and largely with the probable bedding, while the single faults are less regular in their strike, some of them branching. The shear zones cut across the foundation practically at right angles to the length, while some of the single faults make an angle as small as 60° to length of dam.

Third: Mud Seams. Formed along expansion and contraction joints more or less parallel with the surface, these usually end in a conspicuous strike or dip joint, fault or shear zone. At the south end of the dam these mud seams constituted the chief unsoundness and the foundation excavation was carried down to a depth of more than 20' to get below them. At the north end of the dam the lowest mud seam was 8' below the surface and in the main flood plain portion of the Morgan Creek valley they extended to a depth from 3-5' below the solid rock floor.

In the construction of the dam the dip joints which ran with the length of the dam were taken advantage of in sinking the cut-off trench on the upstream side of the foundation. This trench was extended below the mud seams. Of the lines of unsoundness which crossed the

foundation the only ones which gave concern, beside the nearly horizontal mud seams, were the shear zones and the more conspicuous single faults.

In all cases of unsoundness lines crossing the foundation, the cut-off trench was carried down to sound rock and 2" pipes were placed along these lines of unsoundness to extend up through the basal concrete portion of the dam, so that when sufficient weight was superimposed these lines of weakness and possible leakage were strengthened and sealed by concrete grouting.

*Note on a New Eurypterid from the Moscow Shales of New York.* E. N. KJELLESVIG.

The New York Devonian, unlike the Silurian which exhibits a rich eurypterid fauna, has yielded very few species of eurypterids. The known species are referred largely to the genus *Stylonurus*, while the other is referred to the genus *Pterygotus*.

In 1919, Ruedemann reported a new eurypterid from the "Oneonta Sandstone" at Gilboa, N. Y. This species was described as *Pterogotus inexpectans*, and was the first eurypterid of this genus ever found in the Devonian of New York. At a later period, the "Oneonta Sandstone" at Gilboa was found to be Upper Moscow in age.

The only eurypterid of Moscow age, of which the writer has any record, is this same *Pterogotus inexpectans*.

The new species to be described was found by Dr. G. R. MacCarthy in the Upper Moscow Shales near Ludlowville, New York. This form is represented by a single specimen exhibiting a carapace and fragments of the first five tergites. The compound eyes are present; however, one is distorted and moved posteriorly. The total lack of the appendages has made it impossible to ascertain the exact generic position of the specimen. From the rather scanty evidence furnished by the fossil it would seem most probably to be referable to the genus *Eurypterus*, although the possibility of its being a *Stylonurus* must be admitted. It is hoped that the future discovery of more complete material will determine the exact generic position. Until then, the species will be known as *Eurypterus (Stylonurus?) macCarthyi*.

*Industrial Quality of Public Water Supplies of the Coastal Plain.* C. S. GROVE, E. E. RANDOLPH and J. C. McNAIR.

Water is one of the greatest natural resources of any region. The wholesomeness of the waters of this state has been studied and the public



water supplies are kept potable by constant supervision of the Public Health Association.

For several years the Chemical Engineering Department of State College has made special study of the industrial quality of the surface waters of this state and the former results of this work are published in Economic Paper No. 61 of the North Carolina Department of Conservation and Development.

A research study has also been made by this department of the iodine content of North Carolina water supplies.

But potable water does not mean water of high industrial quality. This investigation covers a survey of the industrial quality of the public water supplies of the state in order that industries and consumers may know the suitability of the water for their particular uses, and if not suitable be prepared to correct it for their use. Work has so far been concentrated on the water supplies of the coastal plain. A hundred analyses have been made, many of them duplicates to eliminate varying conditions of the raw water supplies and variations in rainfall. These indicate the industrial quality of the waters of this area.

Supplementing this work on the industrial quality has been the study of the sanitary results, which are being correlated with the work of the Public Health Association. Foremost in this are the fluorides determinations. It has been claimed that fluorides are responsible for the formation of mottled enamel in teeth of adolescents. Results, at present, do not warrant a statement as to the importance of fluorides on the prevalence of mottled enamel.

#### *Symbolic Cubic Forms in Six Variables.* RUTH W. STOKES.

This paper shows that every symbolic form of degree three and class six can be reduced to one of the following canonical forms:

- (1)  $u_1 u_2 u_3 + u_1 u_4 u_5 + u_2 u_4 u_6,$
- (2)  $u_1 u_2 u_3 + u_4 u_5 u_6,$
- (3)  $u_1 u_2 u_3 + u_1 u_4 u_5 + u_2 u_4 u_6 + u_3 u_5 u_6 + u_4 u_5 u_6.$

The above types are non-equivalent. Form (1) is distinguished from the others by having its square zero. Form (2) admits a linear form of rank three, whereas every linear form is of rank one with respect to (3).

#### *Analytic Criteria for Geometrical Conditions.* H. V. PARK.

In this paper I have given analytic representation to some geometrical conditions arising from a study of Geometry.

I have divided it into three divisions: (1) Conditions on conics as

referred to the rectangular Cartesian system of coördinates; (2) conditions on conics as viewed from the projective viewpoint; (3) conditions for united position in the plane and in space; and a few conditions arising from the study of binary forms.

*On the Forms of Equations of Curves.* SIDNEY G. ROTH.

The purpose of this paper was to discuss, without any detailed account, some of the various forms of curves of order higher than the second and to investigate properties which were inherent in those forms. For the first part of the discussion, three of the basic properties of cubics were expounded from the point of form of equation. As for the second part of the paper, six distinct forms of  $n$ -ics were presented and a few properties of these curves were derived by exposition of the equation. The method of approach was sampled after that of Salmon's "*Higher Plane Curves*."

*The Classification of Collineations in the Plane.* E. T. BROWNE.

A collineation in the plane is a projective transformation of a plane of points into itself in such a way that points which are collinear are transformed into points which are collinear. Points and lines whose positions are unchanged are called *fixed* or *invariant* elements of the collineation.

Collineations are classified according to the orientation of the fixed elements. This classification can be made from either the geometric or the algebraic standpoint. Most of the texts on analytic projective geometry adopt the former viewpoint. Those that adopt the latter point of view employ the theory of *elementary divisors*, which is much more elaborate than is necessary for the problem under consideration. Moreover, the average student of projective geometry is totally unacquainted with it at that stage in his mathematical career.

In this paper an algebraic classification is made. However, no use is made of the theory of elementary divisors. On the contrary, the discussion presupposes a knowledge of only the simplest facts from the theory of equations. The classifications is greatly facilitated by the introduction and proof of two new theorems.

This paper is to appear in full in an early issue (probably September) of the *American Mathematical Monthly*.

*Integral Equations with Solutions of Limited Variation.* F. G. DRESSEL.

Calling a function  $K(x, y)$  of class A if it can be written as a continuous function of  $x$  and  $y$  divided by  $(x - y)^\alpha$ ,  $\alpha < 1$ , the paper shows that

the Volterra integral equation of the second kind with a known function of limited variation and a kernel  $K(x, y)$  of class A has a solution of limited variation if

$$\int_y^x K(x, s) ds = \int_y^x T(s, y) ds,$$

where  $T(x, y)$  is also a function of class A.

*Certain Conics Associated with Non-singular Correlations in the Plane.*

L. L. GARNER.

These conics, a point conic and a line conic, are obtained by requiring a point (line) to lie on its corresponding line (point), defined by a correlation of points and lines of a plane. The transformation is reduced to a certain canonical form, and a classification is made according to the rank of the matrix obtained by imposing the conditions for points of a double pair. These latter conditions lead to a characteristic cubic, which is a reciprocal equation, the roots of which may be listed as: (A) 1, 1, 1; (B) 1, -1, -1; (C) 1,  $r$ ,  $1/r$ . Cases (A) and (B) are each divided into two subdivisions. In the first and last cases, the conics are non-degenerate; whereas in case (B) they are degenerate.

*Open Subsets of Non-compact Continua.* JOHN H. ROBERTS.

It is well known that if  $M$  is a compact continuum and  $K$  is a closed subset of  $M$ , then every component of  $M-K$  has a limit point in  $K$ . However, if the requirement that  $M$  be compact is removed, the result does not follow. In the present paper it is shown that if  $M$  is a plane continuum and  $K$  is any closed subset of  $M$ , then at least one component of  $M-K$  has a limit point in  $K$ . An example is given of a continuum  $M$  in euclidean 3-space containing a subcontinuum  $K$  such that every component of  $M-K$  is a continuum.

*The Elongation of a Rubber Band as a Function of the Number of Applications of an Intermittent Stretching Force.* MILTON L. BRAUN.

After a rubber band is stretched it does not return to its exact original length when the stretching force is removed. Furthermore, the stretch produced by the second application of a given force is not the equivalent of that produced by the first application of the same force. The problem under investigation is the determination of the effect of a number of applications of a force, or the stretch produced by the  $n^{\text{th}}$  application of a given force to a rubber band.

In this experiment the band was suspended from a non-abrasive support, a load was slowly (and presumably isothermally) applied, left suspended for one minute at which time the length of the band was recorded, the force was then slowly withdrawn, and after one minute the length again recorded. This cycle of operations was repeated many times. The results show that the stretch due to the  $n^{\text{th}}$  application of a given intermittent force follows a power law,

$$S_n = an^k$$

where  $S_n$  is the stretch involved,  $n$  the number of the application, counting consecutively from the first stretch of the series on a new band.  $a$  and  $k$  are constants depending apparently upon the original length of the band, its cross-section, time elapsing between application and removal of the load, temperature, kind of rubber, its previous treatment, etc. As the equation shows,  $a$  is the stretch produced by the first application of the force under the conditions of the experiment. The constants were empirically evaluated from the consideration that

$$S_n = l_2 - l_1 = f_2(n) - f_1(n) = F(n)$$

where  $l_1$  is the length of the band after the intermittent force had been applied for the given time interval and then removed for that same interval,  $l_2$  its length with the load suspended from it for the specified time interval. Though these lengths are functions of  $n$  it was found difficult to express them algebraically. However, the final function,  $F(n)$ , was found to be remarkably true to the power form. Typical of the equations obtained is the following for an ordinary, five centimeter, one-seventh gram, best grade Para rubber band, at room temperature, with intermittent load of three hundred grams:

$$S_n = 16.36 n^{.66},$$

the average deviation between observed and computed values being less than  $\pm 0.2$  per cent, with each one of the 40 observed points well within one per cent of the computed values. Both  $a$  and  $k$  increase with an increase in the stretching force.

*The Saturation Magnetization of Cubic Cobalt.* F. W. CONSTANT and R. I. ALLEN.

Using an ellipsoid of cobalt, quenched so as to be in the cubic state, and a Weiss electromagnet the magnetization at a given temperature

was measured for increasing fields. Using Weiss' formula for the approach to saturation the magnetization for an infinite field was calculated. This was repeated at various temperatures from that of liquid air to that of boiling water. The saturation magnetization decreased linearly with the square of the absolute temperature. Carrying this proportionality back the intensity of magnetization at absolute zero,  $J_0$ , was found to be 1345 e.m.u. per cubic centimeter. Furthermore, when  $J/J_0$  was plotted against  $(T/\theta)^2$ ,  $\theta$  being the Curie Point, the line obtained coincided with that for iron and nickel, both cubic crystals, but not with the curves for hexagonal cobalt and other non-cubic materials. A satisfactory theoretical explanation of these results has not yet been offered.

*Diamagnetism and Paramagnetism in Intense Fields.* D. H. MOORE  
and F. W. CONSTANT.

Previous measurements on dia- and paramagnetism have been carried only to fields of 22,000 gauss. In the present work a Weiss electromagnet giving over 30,000 gauss and the Gouy method were used. The magnetism of Cu, Ag, Bi, and Pt increased linearly with the field up to 30,000 gauss. This means that the susceptibility of these materials is constant for the fields investigated. A ferromagnetic impurity of less than .004 per cent present in the copper contributed a constant magnetization to the specimen. The iron forming this impurity was calculated to be quite ferromagnetic, with an intensity of magnetization of at least 200 c.g.s. units, and probably considerably higher, depending on how much of the allowed .004 per cent of impurity was present.

H. R. TOTTEN, *Secretary.*

## PROCEEDINGS OF THE ELISHA MITCHELL SCIENTIFIC SOCIETY

OCTOBER 11, 1932, TO MAY 9, 1933

338TH MEETING OCTOBER 11, 1932

W. C. COKER: *The Opportunities for Botanical Study at the Highlands Laboratory.*

In 1931 at Highlands, N. C., a compact and well built laboratory was completed and opened for the use of students of natural history. In August of that year it was dedicated as the Weyman Memorial Laboratory. It is the property of a corporation known as the Highlands Museum and Biological Laboratory. The building has all conveniences such as water, lights, individual work rooms and one large central room, dark room for photographic work, drying apparatus for plants. The building is situated in the woods on the edge of a lake about 7 acres in extent which is owned by the corporation. They own about 10 acres of land surrounding the lake. The building is intended for the use of professors and students engaged in research in any of the natural history subjects. There is no class instruction yet carried on there.

The laboratory is ideally situated for the study of mountain life. The elevation of the building is about 3800 feet and arising immediately around it are mountains with elevations of about 4500 feet to over 5000 feet. The highest Alleghanies are in easy reach by good roads. The forests on the mountain sides and in the coves are not surpassed if equalled by any others in the eastern United States, very large areas being primeval and containing the largest known specimens of several kinds of trees, as birch and hemlock. These magnificent forests offer unsurpassed opportunities for botanizing in any phase of the subject. They are wonderfully rich in fungi of all sorts and present in profusion the beautiful and varied flora of our mountains. In addition to the laboratory lake there are other lakes in and near the town and these have been pronounced unusually rich in aquatic life, both animal and plant. In a congenial environment and delightful climate, the student will find at Highlands both equipment and material for original investigation.

SHERWOOD GITHENS: *The Magnetic Field of a Solenoid Oscillating at Radio Frequencies.*

The laws governing the magnetic field of a solenoid excited by direct current have been known for many years. However, with the increased use of high frequency alternating current, the questions arose: (1), are the directions of the lines of force in the magnetic field of a solenoid excited at radio frequencies the same as those obtained with direct current excitation; and (2), is the distribution of field intensity the same? Upon investigation with specially designed search coils it was found that the magnetic field of a solenoid is similar both in flux direction and distribution to that obtained with a direct current, when a "push-pull" vacuum tube oscillator is employed to furnish the radio frequency current. However, when a "tuned plate-tuned grid" oscillator is used the magnetic field is somewhat different. The maximum field strength occurs not at the middle but slightly toward the "filament" end of the solenoid; and the line-of-force field is slightly distorted, the distortion being of a shearing nature and more pronounced at the "plate" end. It is therefore concluded that when a rapidly reversing magnetic field with uniform and parallel lines of force is required, the middle third of a solenoid excited by a "push-pull" oscillator should be used.

339TH MEETING, NOVEMBER 8, 1932

J. B. BULLITT: *A Comparison of Several Human Species* (Exhibit).

A. S. WHEELER and J. H. WATERMAN: *Cymyl Orange: A New Indicator.*

Aminocymene was converted into its sulfate; salt was dehydrated and sulfonated with fuming sulfonic acid at 165°. The purified sulfonic acid was diazotized and coupled with dimethylanilin. The orange colored dye produced is an indicator. Its formula is  $\text{CH}_3 \cdot \text{C}_6\text{H}_7 \cdot \text{SO}_2\text{OH} \cdot \text{C}_6\text{H}_4\text{N}_2 \cdot \text{C}_6\text{H}_4\text{N}(\text{CH}_3)_2$ . It is crystalline, orange colored and a better indicator than the well known and much used methyl orange. The superiority is due to the fact that the two colors obtained with methyl orange in acid and alkaline solutions show a distinct brown tone whereas cymyl orange gives a pure yellow and pure pink.

340TH MEETING, DECEMBER 13, 1932

H. N. JENKS: *Environmental Influences of the Water-Sewage Cycle* (Lantern).

H. V. WILSON: *Adaptive Behavior of Sponges in Making a Skeleton.*

A Suberites from Chesapeake Bay, recorded as *S. paradoxus* Wilson

but perhaps representing only a phaenotypical variation of a known species, ekes out its spicular skeleton with sand grains which are incorporated in outgrowths extending down into the substratum on which the lamellate body rests. An approach is thus made to the condition present in Phoriospongia and Keratose genera. *Stylotella heliophila* in parts of Beaufort (N. C.) harbor spreads out in thin sheets over a sandy bottom, its attachment to which is accomplished by the incorporation of sand grains instead of by the formation of spicular root-bundles as in some other sponges. This utilization of ready-made bodies with a consequent saving of energy in the process of skeleton formation is far more strikingly illustrated in the two cases on which Professor E. Topsent of Strasbourg has recently published (Arch. de Zool. Expér. et Gén., 68, Notes et Revue: 19). Topsent finds that *Acarus tortilis* when growing on *Geodia cydonium* may use the large projecting oxaeas of the latter instead of building up columns of styles. Again *Anomoclathria opuntioides* sometimes grows on and incorporates a filamentous alga, in which case primary skeletal fibers are not formed and the secondary skeleton is arranged with respect to the algal branches as if these were the primary fibers.

Such instances of adaptive alteration of morphogenetic behavior are allied to what is known as "regulation" of embryogenic processes and probably at bottom are not far from the "purposive reflexes" of higher animals. We may thus look on them as processes which represent steps in the evolution of that great complex which in the higher animals is referred to as "intelligence."

#### 341ST MEETING, JANUARY 10, 1933

H. D. CROCKFORD: *Some Studies in Strong Electrolytes.*

1. A mathematical solution of the problem of the calculation of the mean distance of closest approach of the ions in dilute solutions of strong electrolytes has been made. This quantity has been obtained as a function of quantities depending on the nature of the individual ions.

2. The activity coefficients of lead chloride in solutions of cadmium nitrate have been determined at different concentrations. These data have been employed to check the equation obtained from the solution in (1).

H. N. DEWICK: *The Relative Effectiveness of Visual and Auditory Presentation of Advertising Material.*



## 342ND MEETING, FEBRUARY 14, 1933

W. C. GEORGE. *Some Phenomena of Self Adjustment in Ascidians.*

Experimental material: *Styela plicata*, a large simple ascidian with thick tough tunic. If either the oral opening or the atrial opening be closed by tying a string around the siphon, a new opening is formed just below the ligature within about twenty-four hours. Subsequently the characteristic feature of the typical siphon are formed about the new opening. The reactions leading to the formation of new openings need not be looked upon as purposive. It appears that the stimulus of the ligature, supplemented probably by deficiency in nutrition and respiration, causes the mantle of the siphon to be pulled loose from its attachments to the siphon tunic and to be withdrawn into the space below the ligature. Here it acts as a nozzle to direct against a spot on the tunic below the ligature the force of the water pressure resulting from further contractions of the mantle musculature. This pressure results in a thinning and a rupture of the tunic at the point where the pressure is applied.

J. A. ALEXANDER: *Geo-magnetic Surveying.*

The magnetic method of geophysical work deals with variations of the direction and intensity of the earth's magnetic field.

Using an Ascania vertical magnetometer, we measured the vertical component of this field. Scale readings were directly proportional to the vertical field strength. By plotting stations on a map and drawing lines of equal variations from an arbitrarily chosen base or by plotting profiles from this base, it was possible to map geologic structures containing different amounts of iron in adjoining areas.

The method proved especially efficient in tracing basic dikes in Triassic sandstone, these being shown by higher readings even where covered by later stream laid material. Apparently, it is quite suitable to general structural mapping in Triassic areas, the piedmont and some parts of the coastal plain and may well become a valuable aid to the structural geologist working in these areas.

## 343RD MEETING, MARCH 7, 1933

J. H. PRATT: *Mineralogical Notes on North Carolina Minerals.*

Of the one hundred and forty-one manganese minerals that are now recognized, fifteen have thus far been found in North Carolina. Six of these minerals were unknown in North Carolina until 1931, when the manganese vein or deposit at Bald Mountain, Alleghany County, was

opened up. These minerals are alleghanyite and galaxite, new minerals, rhodonite, tephroite, bementite, and neotocite. Another mineral new to North Carolina was also found in this vein: namely, grunerite  $(\text{FeMg})\text{SiO}_3$ , one of the Amphiboles. Galaxite belongs to the spinel group. This is the second new mineral from North Carolina to be added to the spinel group, the other being mitchellite,  $\text{FeOAl}_2\text{O}_3 \cdot \text{MgOCr}_2\text{O}_3 \cdot 2\text{MgOAl}_2\text{O}_3$ .

The spinel group of minerals represented by the general formula  $\text{RO} \cdot \text{R}_2\text{O}_3$ , where  $\text{R} = \text{Mg} \cdot \text{Fe}$ ,  $\text{Zn}$  and  $\text{Mn}$ ; and  $\text{R}_2 = \text{Al}$ ,  $\text{Fe}$ ,  $\text{Cr}$ , and  $\text{Mn}$ , is a most interesting group of minerals and several more members may be expected to be added to this spinel group.

A study of the composition of several of the spinels such as dysluite, a variety of gahnite,  $(\text{Zn} \cdot \text{Fe} \cdot \text{Mn})\text{O} \cdot (\text{Al} \cdot \text{Fe})_2\text{O}_3$ , of franklinite,  $(\text{Fe} \cdot \text{Zn}, \text{Mn})\text{O} \cdot (\text{Fe}, \text{Mn})_2\text{O}_3$ , of jacobsonite,  $(\text{Mn}, \text{Mg})\text{O} \cdot (\text{FeMn})_2\text{O}_3$  and of picotite  $(\text{Mg}, \text{Fe})\text{O} \cdot (\text{Al}, \text{Fe}, \text{Cr})_2\text{O}_3$ , indicates that there are several spinel molecules occurring in combination which thus far have not been found in nature, such as  $\text{MgO} \cdot \text{Fe}_2\text{O}_3$ ;  $\text{MgO} \cdot \text{Mn}_2\text{O}_3$ ;  $\text{MgO} \cdot \text{Cr}_2\text{O}_3$ ;  $\text{ZnO} \cdot \text{Fe}_2\text{O}_3$ ;  $\text{ZnO} \cdot \text{Mn}_2\text{O}_3$ ;  $\text{FeO} \cdot \text{Mn}_2\text{O}_3$ , and  $\text{MnO} \cdot \text{Mn}_2\text{O}_3$ . Some of these will undoubtedly be found as minerals. The  $\text{MgO} \cdot \text{Cr}_2\text{O}_3$  molecule forms a considerable percentage of the composition of the spinel, mitchellite.

There are 55 minerals classified as uranium minerals, of which ten have thus far been found in North Carolina. Of these phosphuranylite and clarkeite were first identified in this state. Clarkeite has been formed from the alteration of uraninite. This mineral was formerly supposed to be gummite which was considered the first alteration product of the uraninite. Now the series is shown to be from uraninite through clarkeite, gummite, to uranophane.

During the summer of 1932 very beautiful crystals of torbernite were found at one of the feldspar mines near Spruce Pine. These are flat, tetragonal crystals from one to four mm. wide, with the basal plane very prominent, and the prism  $a(100)$  slightly developed as are also two pyramids probably  $x(105)$  and  $y(102)$ .

W. DEB. MACNIDER: *The Response of the Liver to Large Amounts of Ethyl Alcohol* (Lantern).

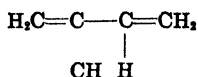
344TH MEETING, APRIL 11, 1933

T. F. HICKERSON: *Continuous Beams with Variable Sections*.

B. L. JOHNSON: *A Study of the Aging of Rubber*.

The research discussed was one concerning the physical and chemical changes which take place when the rubber is aged. By the aging of rubber is meant its deterioration; for example, increase in tackiness, loss of tensile strength and its finally becoming cracked and brittle.

The structure of rubber itself suggests several theories as to the mechanism of its aging. The more important ones were discussed. Rubber is composed of polymers of isoprene which has the formula



One theory of deterioration is that oxygen attacks the rubber by addition at the double bond.

Another method of deterioration of the rubber might be a depolymerization or a cleavage in the rubber micelle. The possibilities of this reaction were studied by means of x-ray photographs. The x-ray interferences of crude rubber appear only at a minimum elongation of 80 to 100 per cent. Bary and Hauser have explained the formation of x-ray patterns upon elongation of rubber on the basis that, in the unstretched rubber, crystalline aggregates or fibers exist but are swollen by the lower polymers so that it is impossible for them to give a diffraction pattern. When the rubber is rapidly stretched, the lower polymers are expelled leaving the regularly oriented crystalline aggregates which produce the x-ray diffraction pattern. From this view, the fact that as the time of oven aging is increased the elongation necessary to produce an x-ray pattern of rubber must be increased may be interpreted as meaning that there had been a depolymerization and the greater elongation was required because the concentration of lower polymers to be expelled had been increased during aging. However, this work indicates that there is the same amount of depolymerization in both the rubber with and without antioxidant. This indicates that other factors (perhaps oxidation) than the shift to the polymers of the lower type caused the the greater decrease in tensile strength found in the case of the antioxidant free rubber.

The fact that the position and width of the diffraction lines stays the same at various elongations indicates that the decrease in intensity of the diffraction lines upon aging is due to the smaller number of crystal aggregates or micelles and not to a variation in the size of a constant number of micelles.

## 345TH MEETING, MAY 9, 1933

E. T. BROWNE: *An Unsolved Problem in Mathematics.*

COLLIER COBB: *North Carolina Granites in Art.*

The following officers were elected for the year 1933-34:

President—J. W. Lasley, Jr.

Vice-president—K. H. Fussler.

Secretary-Treasurer—C. D. Beers.

## EARLY MAN\*

By J. B. BULLITT

### PLATE 1

Last spring this Academy saw fit to select as its president one of its obscure members instead of one of its active leaders. Perhaps this departure from usual customs was intended as a sort of scientific experiment, a "test by trial and error." Though the error may have been great, I am deeply sensible of the honor conferred upon me, and I wish to give voice to my sincere appreciation of this expression of your confidence.

In this body the secretary does all the real work, and the burdens of the president are not heavy. His chief duty is the preparation of a presidential address. Tradition decrees that such a discourse should be either an erudite review of some subject or a presentation of important original work. This has put me in a quandary; for I am lacking in erudition, and the only originality to which I can lay claim is a rather liberal share of original sin. In this staunch Presbyterian community, this frank confession may explain, even if it does not excuse, some of the heretical doctrine which I shall promulgate tonight. In the matter of originality, however, I take some comfort in the saying of some one that it consists not so much in saying things that have never been said before as in saying them to people who have not heard them before. Therefore, I am basing the first part of my talk upon the first chapter of a great Book, whose name is on the tongue of everyone and whose interpretation is the subject of much controversy, but whose actual contents are none too familiar to most of the disputants, Modernist and Fundamentalist alike. The remainder of the talk will deal with a few pictures of some of our kin folk, whose names are doubtless known to all of you, but whose faces are perhaps strange to most of you. I say *kin folk* advisedly, for my Kentucky birth and Virginia ancestry bid me to claim kin, not only with those who are near in blood and high in favor but also with distant and obscure and even disreputable relatives.

"In the beginning God created the heaven and the earth. And the

\* Presidential Address before the North Carolina Academy of Science, at Davidson College, May 5, 1933.

earth was without form and void," a great planitesimal cluster or perhaps a nebula, in either case a vast quantity of star dust, shapeless and lifeless, whirling through endless space. When, after long ages, the star dust fused into a solid ball, the heat of the young earth kept it shrouded in the gloom of a heavy fog. "And darkness was on the face of the deep. . . . And God said, Let there be light;" and a faint glow came through the thinning mists and divided the day from the night. "And the evening and the morning were the first day" or the Astronomic Era.

And God divided the waters from the waters; and fogs rose into cloud banks, and rains fell to envelop the earth with a shallow sea. "And the evening and the morning were the second day" or the Hydro-spheric Stage of the Pregeologic Era. No geological record remains to tell us anything concerning the conditions on the earth during these first two immensely long Eras or Days.

"And God said, Let the waters . . . be gathered together . . . and let land appear;" and lands rose from the sea, and sank and rose, again and yet again. "And God said, Let the earth bring forth grass;" and in the swampy ooze came the first plants, and some preyed upon others and became the first animals; and they increased and multiplied, each after its kind. Time has wiped all clear record from the rocks; but bits of graphite and chalky stone tell us that life was there. "And the evening and the morning were the third day" or the Archeozoic Era.

The clouds were still heavy and the light was dim, but God rolled back the clouds, and the sun shone through to rule the day and the moon and stars to rule the night; and they became signs for the days and the seasons. Ages passed, and living things increased in number and in kind. But lands sank and rose; and storm and flood and ice and volcanic fires bit into the rocks, and the tales that they tell are blurred until we can read them but darkly. "And the evening and the morning were the fourth day" or the Proterozoic Era.

"And God said, Let the waters bring forth abundantly the moving creature that hath life and the fowl that may fly above the earth." "And the evening and the morning were the fifth day" or the Paleozoic and the Mesozoic Eras, a vast and inconceivable stretch of time. The rocks testify that from the very beginning of that day both sea and land swarmed with life. All the great phyla were already present. And as the ages passed, old species died out and new forms took their place; and some became Trilobites and Graptolites and Cephalopods and Fishes and Reptiles and flying things, the progenitors of our Birds; and great Ferns and Conifers covered the earth and laid the beds for our coal and oil fields.

"And God said, Let the earth bring forth the living creature after his kind, cattle and creeping thing, and beast of the earth after his kind." And He made man to have dominion over them, the fish and the fowl and the beast and the creeping thing. And man was naked and unashamed; and he was "a mighty hunter before the Lord." He followed the bear and the wolf to their dens and fought them for the caves where he made his home. "And the evening and the morning were the sixth day" or the Cenozoic Era, the Tertiary and Quaternary Ages, the Ages of Mammals and of Man.

I have abbreviated and paraphrased and interpreted the Biblical text; but I have done this reverently, and have omitted none of its essential record nor added anything not in accord with it. Across the gap of

## CHART I

First day....	<i>Astronomic Era</i> .....	No geological record remains.
Second day...	<i>Pregeologic Era</i> .....	{ No geological record recognizable. Probably no life existed.
Third day....	<i>Archeozoic Era</i> .....	{ Only igneous and metamorphosed rocks. Obscure evidence of life.
Fourth day...	<i>Proterozoic Era</i> .....	{ Rocks similar to Archeozoic Era. Scanty but definite signs of life.
	<i>Paleozoic Era</i> .....	Sedimentary as well as igneous and metamorphosed rocks. Abundance of fossils. All the great phyla are represented.
Fifth day.		
	<i>Mesozoic Era</i> .....	{ Rocks similar to above Era. Fossils abundant. Reptiles dominant. First primitive mammals appear.
Sixth day {	<i>Cenozoic Era</i> {	{ Tertiary Age—Mammals dominant.
		{ Quaternary Age—Man dominant.

thirty centuries the modern scientist can clasp hands with this ancient Hebrew Evolutionist. He lacked our store of Astronomical and Geological data; yet he had the keen perception to sense the main outlines of the story of the physical development of the earth and the slow evolution of living things from the simple to the more complex, culminating in man. It is interesting to note that he devotes only two "days" to the time concerning which we have clear geological record of life upon the earth, while he gives four "days" to those immensely longer preceding ages whose geological record has been so largely erased. It is hazardous but tempting to speculate as to what this ancient writer may have known of geology and paleontology. We are prone to think that our recently acquired science is new; but we sometimes meet surprises when we come face to face with the knowledge and wisdom of our forefathers.

We call man the highest of all the animals. In reality he is no more highly evolved than hosts of others, but the directions of specialization are different. The horse's hoof is more highly evolved than our hand; it departs more widely from the primitive pattern. The one is adapted for rapid locomotion on hard ground, the other for delicate manipulation. The armadillo's armor, the butterfly's wing, the ant's antenna are examples of specialization higher than that in our corresponding parts. Even in the nervous system, the fish's olfactory lobe is evolved far beyond that of man. Man's great specialization is in his fore-brain. This, with his wonderful manipulating hand, gives him his dominance over the rest of creation, and enables him to be the most wantonly destructive of all God's creatures. Man appeared as a mere speck of cloud upon the late evening sky of the earth's long day, but before night has fallen, that cloud has spread from horizon to horizon and the resultant deluge is fast sweeping away the former abundant life of the world.

We do not know where nor how nor when man originated. The weight of present evidence places his probable origin in central Asia, but like all scientific theories this view is subject to change. The mechanism of evolution and the stages by which it occurred are still matters of speculation, with ample room for wide differences of opinion; but all students of biology and geology and most other well-informed people accept evolution as a fact and believe that men, apes, and monkeys sprang from a common stem.

This common stem arose some millions of years ago, apparently in the Eocene Period of the Tertiary Age of the Cenozoic Era. That would be the morning of the Biblical "sixth day." The humanoid branch probably separated from the other primates near the middle Tertiary (Oligocene or Miocene Period), the forenoon of the "sixth day." Our first clear evidence of anything that might be called man occurs in the late Tertiary (Pliocene), the afternoon or evening of the "sixth day." In the sands and gravels of that time, before the beginning of the great glacial epochs, about one million years ago, we find stone implements that must have been shaped by intelligent direction. From then until the present day, the chain of evidence of *man's handiwork* is unbroken, though his skeletal remains are few and fragmentary until comparatively recent times, a few thousand years ago. From these scanty threads we try to weave the web of his early life. There are great gaps in the fabric and every part is imperfect.

We regard all modern races of men as belonging to a single species, in spite of wide differences that would doubtless be considered of specific



character in any other animal. We have unmistakable skeletal evidence, however, that in the past at least five or six distinct species have existed. Moreover, fragments, too imperfect for positive classification, seem to represent still other species. From an evolutionary standpoint we should like to trace the descent from a distinctly simian ancestor down through higher and higher humanoid and human forms to the present *Homo sapiens*, but our data are insufficient. In the case of some of these species, our evidence is limited to merely a few bones or parts of bones of only one or two individuals. Skeletons and parts of skeletons have been found which represent varying *degrees* of evolutionary progress, but we can not call them definite *stages* of development from the

CHART II

Cenozoic Era, sixth and seventh days	Tertiary Age	Eocene Period	{ Probable beginning of common primate stem.
		Oligocene Period	{ Humanoid branch probably separated from common primate stem.
		Miocene Period	
		Pliocene Period	{ Primitive stone implements give first evidence of man's existence.
	Quaternary Age	Pleistocene Period (1,000,000 years)	{ Four Glacial Epochs. Abundance of man's implements. Fossils of several human species, including modern types.
		Holocene Period Postglacial (20,000 years)	{ Modern man: all other species are apparently extinct, except possibly Rhodesian Man.

prehuman to the human, nor from the primitive human to the modern human. They exhibit in varying measure the physical features and presumably the mental traits that distinguish modern men from apes. I propose to show tonight pictures of some of these fossil skulls, and to call attention to a few of their anatomical characters.

These can be best understood by first comparing these same characters in modern man and in one of the great anthropoids.

1. Compare the bowed spine, crouching posture, and semiquadrupedal gait of the gorilla with the sinuous spine, erect posture, and free bipedal gait of man. Associated with these things are differences in the form of the bones of the pelvis and the lower extremities, especially the articulations at hip, knee, and ankle.

2. Note the articulation of spine and skull. In man, this point is practically beneath the center of gravity of the head, giving it an easy balance upon its support (fig. j); in the gorilla this joint is far behind the center of gravity (fig. J), throwing the head forward and demanding powerful muscles in the back of the neck to prevent the chin from falling upon the chest.

3. The essential basis for the above difference lies in the comparative size of the face and the brain case. The gorilla's long, massive jaw and projecting face overbalance his small brain (fig. J); man's face is retracted and his jaw is relatively small, while his brain is more than twice the size of that of his nearest and largest simian cousin (fig. j). A man of 150 pounds weight has a brain volume of 1400 or 1500 cc., while a 300-pound gorilla's brain has a volume of about 600 cc.

4. As a corollary to this brain development, the human skull is broad between the temples, the forehead has a steep upward rise, and the vertex is high above the ears (figs. *H* and *h*). The gorilla's low forehead throws his brow into strong relief. Moreover, the attachments of his tremendous muscles of mastication require a great bony buttress across his brows and a strong crest along the top of his head; in spite of the narrowness of the actual brain case, the face is broad through the cheeks and the whole head is thick through the temples because of the huge size of these muscles (figs. *I* and *i*). The corresponding muscles in man rise scarcely more than half way up the side of the skull to the faintly marked temporal ridge.

5. Compare the relatively small teeth of a man with the great molars and formidable, interlocking canines of the gorilla (figs. *H* and *I*). Man's chin juts forward, while that of the ape can scarcely be said to exist. (It is interesting to note that the Latin word *mens* means both mind and chin.) When we look at the lower jaws from above, we see that in man the sides flare widely and the row of teeth forms a curve like a wide open horse-shoe or like a "V" with a very rounded point, while in the ape the sides are compressed and the row of teeth forms a curve like a mule's shoe or very narrow "U," and across the closed portion of the "U" runs a strong bony bar (the Simian shelf). This difference bears an important relation to the mobility of the tongue in speech (figs. *h* and *i*).

These are only a few of the many differences.

In showing these skulls I shall not follow the chronological order of their discovery nor the chronology of their existence on earth. I choose an order of convenience in demonstrating some of the anatomical

developments. Our present knowledge of prehistoric chronology permits only the very roughest sort of approximation. A few thousand years, in fact, in the case of the older specimens, a few hundred thousand years mean nothing to us. For brevity's sake I may speak somewhat dogmatically in terms of years, but at best these estimates of time are merely crude guesses.

No true link between man and the other primates (apes and monkeys) has ever been discovered. Neither has a true link between ape and monkey been found, but we cannot escape recognition of the kinship.

#### PITHECANTHROPUS

The most primitive human or humanoid being as yet discovered is the *Pithecanthropus erectus*. Our knowledge of him is limited to merely a few parts of a single individual—a skull cap, a portion of a lower jaw, three teeth and a thigh bone (femur). These were discovered about 40 years ago in Java by Dubois, a Dutch army surgeon. The femur is quite humanoid, straight, slender, and of a length to suggest a stature of perhaps 5 feet, 6 inches. The articular surfaces indicate that he could walk erect or nearly so. A little scientific imagination, using the skull cap and fragmentary jaw as guides, has permitted a reconstruction of the whole skull, which is probably fairly accurate. This shows a strongly projecting shelf-like brow, an extremely low, retreating forehead, and a marked narrowing through the temples (figs. A and a). The jaw and teeth are strikingly ape-like. The brain volume was about 900 cc.—50 per cent larger than that of any ape, but about one third smaller than that of the average modern man. The interior of the skull shows an impression that indicates some development of Broca's convolution—hence probably some power of speech. Whether he made implements or knew the use of fire we have no way of determining. He lived during the early Pleistocene Period, probably about 700,000 years ago; but how much earlier his race began and how much later it survived is totally unknown. Whether we call him a very superior ape or a very inferior man, his facial lines would hardly be called beautiful, and many persons might feel a distaste for calling him "grandfather" or even "cousin."

#### SINANTHROPUS

Early Pleistocene deposits, probably of about the same date as the *Pithecanthropus* gravels, fill certain deep clefts in the hills about 25 miles from Peking. Excavations under the auspices of the Geological

Survey of China have yielded in the past decade two nearly complete skulls, numerous teeth and portions of several lower jaws. Bones of the trunk and the extremities are lacking, except for a portion of a clavicle and a few of the small bones of the hand and foot. Reconstruction of the complete skull has not yet been accomplished, and the volume of the brain has not been determined. This cast of one of the skulls (figs. *B* and *b*) (supposedly an adolescent male) was made before it had been entirely freed from the hard imbedding travertine. This shows a jutting brow, a low receding forehead and narrow temples. The jaw is massive, with deficient chin, but the teeth are distinctly human. Authorities who have studied merely the photographs are divided in opinion as to whether this creature represents a new species or is merely a variety of *Pithecanthropus* or a variety of *Neanderthal man*. Mr. Pei (Chinese), Père Teilhard (French) and Dr. Black (Canadian) who made the excavation and have published detailed studies of the bones hold that he constitutes a distinct species, higher than *Pithecanthropus*, lower than *Neanderthal man*. It is thought that his hand was human and his foot rather ape-like, but we lack material for positive conclusions concerning his trunk and extremities.

Associated with these bones are large numbers of rude implements of quartz and quartzite, bone and horn—knives, scrapers, choppers, daggers, hammers, etc. Great beds of ash and cinders, whose location and disposition exclude the possibility of volcanic or other natural agency as a cause, testify that *Sinanthropus* knew and used fire. Certain human long bones, cracked by stone implements and charred by fire, suggest cannibalism. These implements and these hearths are not the most ancient that have been discovered. The Tertiary implements in Eastern England are older, but this Chinese culture is the oldest that has yet been found in actual association with the bones of its makers. Our knowledge of this early man is limited to the finds at this single site. We do not know where else he roamed, nor when his race began or ended. Probably he was a contemporary of *Pithecanthropus* and perhaps also of *Pitldown man*.

#### NEANDERTHAL MAN

About 85 years ago a very primitive skull was found in a cave at Gibraltar. It attracted some interest and finally found its way into the Museum of the Royal College of Surgeons in England; but its real significance was not appreciated until half a century later. It is actually the earliest find of the *Neanderthal species*, the type skeleton of which was

discovered in 1857 in the Neanderthal cave near Düsseldorf. Since then the remains of more than 150 individuals of this race (infants, adolescents, and adults) have been unearthed. Some of these skeletons are practically complete and in fairly good state of preservation, and in many cases their implements and ornaments accompany their bones. So we have been able to learn much of both his bodily structure and his fairly high culture, the so-called Mousterian. He occupied the greater part of Europe, at least a part of Western Asia and probably Northern Africa. We know that he lived during the Third Interglacial Epoch in Europe and survived through many thousand years of the last or Fourth Glacial Epoch. The Mousterian culture extends back at least half a million years, and perhaps this species of man was equally ancient, though no human bones have been found associated with the artefacts in those earlier millenia. Apparently he became extinct when modern types of man came into competition with him.

Modern man comprises widely varying racial types. So, too, the Neanderthal species had numerous variants. I have chosen for demonstration tonight one of the best preserved and best known examples, excavated about 25 years ago by the Abbés Bouyssonie and Bardon, in a small cave near La Chapelle aux Saints, Corrèze, France (figs. C and c). The beetling brow, extremely retreating forehead and low cranial vault are evident at the first glance; it is also relatively narrow through the temples; but none of these features is nearly so extreme as in *Pithecanthropus*. The head is large. The whole brain is fully equal in size to that of modern man, though the fore-brain seems relatively smaller. The face projects well forward. The jaw is long and heavy, and the chin is cut back in a curve that is intermediate between that of modern man and the ape. A semblance of the simian shelf is present, which may be seen in this photograph (fig. c). The teeth are large and primitive though distinctly human. In spite of the popular notion that all primitive men have sound teeth, that ancient gentleman had lost most of his, so you can see only two old snags in the picture. The great muscles of mastication rose high on the side of his head, and their thickness caused a great bulging of the zygomatic arch on the side of his cheek. The articulation with the spine is set far back, though not nearly so far as in the ape. Nevertheless, his head must have slumped forward most ungracefully. His body was massive, the arms and legs short, his hands and feet huge, but his forearms relatively slender. He was but a few inches above five feet in height, and the articulations of the lower extremities show that his walk was a slouch with knees partly

bent. His portrait is not attractive, but he had the skill to make superb flint implements, he had the intelligence and courage to kill the mammoth and the cave bear, his aesthetic development led him to adorn himself with necklaces and other ornaments, and some of his moral and religious ideas are suggested by the care and ceremony with which he buried his dead.

#### RHODESIAN MAN

A remarkable skull was discovered twelve years ago in Rhodesia. A mine shaft, sunk from the top of Broken Hill, penetrated the roof of a cave. The cave was filled with sediment and débris, containing bones of many modern species of animals, bones of two or more individuals of modern human type, and this extremely primitive skull. No one trained in paleontology was present. No record was made of the stratigraphy or the exact positions of the various bones. The skull alone (figs. *D* and *d*) would be regarded as certainly very ancient, probable early Pleistocene or perhaps Pliocene; the other bones, if found alone, would equally certainly be regarded as modern. Speculation on the subject is still active. Is it possible that an exceedingly primitive species of men survived in Africa until comparatively modern times, contemporaneous there with modern man and modern animals? Do a certain modern type of femur and tibia and fragmented hip bone which were found nearby actually belong to the individual who bore this strange skull? A few years ago every anatomist would have answered this second question in the negative. But the recently proven association of the human Piltdown skull with the ape-like Piltdown jaw makes us cautious. We do not know. We have learned, however, that in some human species some very primitive structures have been retained while other structures have evolved highly.

The general contours of the skull (figs. *D* and *d*) are somewhat Neanderthaloid. The projecting brow is even heavier than in the Neanderthals, but it has a peculiar angular character not present in any other type of man. The forehead is low and receding and the vault is low. The lower jaw was not found. The face is somewhat projecting, though not extremely so. The articulation with the spine is not so far back as in Neanderthal men. From a pathological standpoint he is quite modern; for his teeth are frightfully decayed, he had pyorrhoëa with large abscesses in the bone, and he had mastoiditis with a resultant intracranial abscess, which eroded through the temporal bone. Probably this abscess caused his death.

## EOANTHROPUS (PILTDOWN)

Perhaps the most interesting, certainly the most incongruous and most puzzling of all known human fossils, is the one found some twenty odd years ago at Piltdown Common, in Sussex, England. Under peculiar circumstances which time does not permit me to relate now, an English lawyer, Mr. Dawson, with the assistance of Dr. Smith Woodward and Père Teilhard, rescued from destruction by workmen some fragments constituting a large part of a human skull. Fortunately some of the parts that are missing on one side of the head are represented on the other side, and vice versa. By fitting the pieces together and by duplicating the missing parts of one side by the corresponding parts that are present for the other side, Dr. Smith Woodward reconstructed the skull (figs. *E* and *e*). Later Sir Arthur Keith corrected some errors in orientation of the fragments and made another reconstruction which is almost certainly a close approximation to the original head.

The result is a most amazing combination. The bones are thicker than in any other known human skull, except perhaps *Sinanthropus*, but the form is strikingly modern. This picture (fig. *E*) shows that the brow is not prominent; the forehead is slightly rounded but is fairly steep and reasonably high; there is good breadth through the temples; and if we accept Sir Arthur Keith's reconstruction, the size of the brain is equal to, or nearly equal to that of modern man. Combined with this well shaped head, we have a lower jaw and teeth that might well pass for those of an ape; the rows of molars and premolars on the two sides are practically parallel, the canines are large and interlocking, the jaw is long and massive, the chin is cut away and the simian shelf is well defined (fig. *e*).

For a long time many authorities contended that this was merely an accidental association, a chimpanzee's jaw imbedded in the gravel near a human skull. This would be a striking coincidence, since no other human jaw was found, nor were any other chimpanzee bones. About two years later, at about two miles distance from the Piltdown site, some small fragments of another skull of identical character were found. These showed the same thickness of bone, the same ape-like jaw and teeth; and no other chimpanzee bones were present. Such a double coincidence of association of a human skull with an ape's jaw is unbelievable. So it is now accepted that this peculiar race of men had evolved far as regards skull and brain while still retaining a simian jaw.

The first of these two skulls was recovered from the lowest layer of a four foot, stratified gravel deposit. In the upper strata of this gravel,

flint implements were found belonging to cultures (Chellean or Pre-Chellean) not later than the middle Pleistocene. No unmistakable implements occurred in the stratum with the skull, but there were present some bones of Pliocene mammals. These last were more or less broken and "rolled," that is to say worn and battered in the rushing waters that deposited the gravel. The skull itself shows no evidence of rolling. Its chronology is uncertain, but the above facts indicate that it is at least as early as the second interglacial epoch, probably as early as the first interglacial, and possibly it may have been pre-glacial. Piltdown man may have been a contemporary of Pithecanthropus and Sinanthropus. We have no bones of the trunk or extremities to tell us his stature and figure.

Our data regarding four of these species (Pithecanthropus, Sinanthropus, Eoanthropus, Rhodesian) are limited in each case to finds in a single locality. Moreover, these localities are widely separated. This makes comparative chronology uncertain. It also leaves us ignorant of the geographical range of each species, ignorant as to the degree of kinship and the extent of contact that existed between them or between any of them and other species of man, such as the Neanderthal and modern types. We have ample ground for profitable and constructive speculation but little ground for definite conclusions.

#### CRO-MAGNON MAN

The modern species of man appeared in Europe during the height of the last or fourth glacial epoch. This was perhaps 75,000 years ago. Their cultures (Aurignacian, Solutrean, Magdalenian) were quite different and of a higher character than that of the Neanderthal Men (Mousterian), and probably they were largely responsible for the extinction of the latter. They were the famous cave artists of France and Spain, whose mural drawings constitute one of the most interesting studies in the whole subject of Pre-history. Just as we have today various races of men, so in that glacial day there were various physical types. Some had a strong resemblance to our present negroes, some bore a likeness to the Esquimo, some were much like modern white men. There are various points of difference between them and men of today, but they distinctly belong in the category of *Homo sapiens*.

The skull I have chosen for demonstration is that of the "Old Man of Cro-Magnon" (figs. *F* and *f*). The construction of a railroad along the bank of the Vézère River, near the village of Les Eyzies, in Southern France, unearthed several skeletons under an overhanging cliff. The



"Old Man" was large of body and possessed a head which any modern man would be proud to carry upon his shoulders. The brain volume (over 1600 cc.) is well above the modern average. He may have been a savage hunter but the steep, high forehead, the high vault and the breadth through the temples indicate fine intellectual capacity. The high, narrow, aquiline nose, the strong jaw and the prominent chin suggest a dominating, perhaps a domineering, personality. He must have been an exceptional individual, or it is not likely that in that strenuous Stone Age civilization he would have survived the nearly three score years which the condition of his cranial sutures seems to give as his age. A large depressed fracture on his forehead, entirely healed, tells the story perhaps of some exciting political or domestic altercation, from which he did not emerge unscathed.

## CHART III

Pleistocene Period (1,000,000 years)	First Glacial and Interglacial Epochs	{ Pithecanthropus Sinanthropus
	Second Glacial and Interglacial Epochs	{ Piltdown Man? Galley Hill Man?
	Third Glacial and Interglacial Epochs	Neanderthal Man
	Fourth Glacial Epoch. . . . .	{ Neanderthal Man Cro-Magnon Man Modern Man
Holocene Period (20,000 years).....	Postglacial Epoch. . . . .	{ Rhodesian Man? Modern Man

The student of human evolution must ask the questions, "How old is modern man?" and "Did he descend from any of the known fossil species?" and "What genealogical relation do the various fossil species bear to each other?" Answers to these questions must be largely speculative. We cannot with certainty or even with reasonable probability trace a definite ancestral connection between any of them. The form of skull of Pithecanthropus and Sinanthropus suggests the possibility of descent of the latter from the former, but we lack the necessary data for any more positive statement. The possibility that they may have been contemporaries militates against that conclusion. Comparison of the Neanderthal skull with those of Pithecanthropus and Sinanthropus offers the possibility of descent of the first named from either one of the others. The Sinanthropus stone culture might well be the forerunner of the Neanderthal (Mousterian) culture. Moreover our limited knowledge of their chronology, Pithecanthropus and Sinanthropus

apparently antedating the Neanderthal by several hundred thousand years, makes the descent seem possible, but that is as much as we can say. On the other hand the straight slender femur of *Pithecanthropus* seems more modern than the heavy, bowed bone of Neanderthal man, which seems to exclude the former from the ancestral tree of the latter.

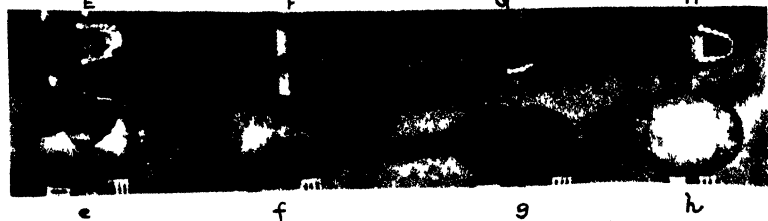
The incongruous combination of the Piltdown skull and jaw (figs. *E* and *e*) seems to exclude him from the direct genealogical line of either of the above named three, though he might possibly be the ancestor of modern man. On the other hand *Pithecanthropus* or *Sinanthropus* might be our progenitor; but Neanderthal man could not be, for his period of existence overlapped that of modern man in Europe. It is even possible that modern man extends back to the days of *Pithecanthropus* and *Eoanthropus* (Piltdown), though we lack satisfactory evidence to such an effect. Practically all paleontologists and prehistorians reject such an idea. A few specimens of definitely modern type have been discovered in very ancient strata (middle or early Pleistocene). Proof that these skeletons were contemporaneous with the deposits cannot be established. It is contended that these must have been intrusive burials within relatively recent times—that is, within the past few thousand years. In the case of the Galley Hill man (figs. *G* and *g*) (found near London in 1888) and the Castenodolo woman (found in Northern Italy in 1880) the entire skeletons were found with the bones still in position. Except in cases of regular burial, the bones would usually be more or less scattered; and it is hard even for prehistorians to believe that the custom of burial is so ancient. The main argument, however, is based upon the inability to believe that modern man can have such great antiquity. A few years ago the same theoretical argument denied the existence of any type of man in preglacial or even in glacial days. Possibly future discoveries may prove *Homo sapiens* to be as old as *Pithecanthropus*.

## PLATE 1

## PROFILE AND VERTICAL VIEWS OF SKULLS

*A* and *a*, Pithecanthropus (reconstruction); *B* and *b*, Sinanthropus (still partly embedded in travertine matrix); *C* and *c*, Neanderthal Man (Chapelle aux Saints); *D* and *d*, Rhodesian Man (Broken Hill); *E* and *e*, Eoanthropus or Pilt-down Man (Smith Woodward reconstruction); *F* and *f*, Cro-magnon Man; *G* and *g*, Galley Hill Man; *H* and *h*, Modern Negro; *I* and *i*, Gorilla; *J*, Gorilla; *j*, Modern White Man.

PLATE 1





# ABNORMAL DENTITION IN RAYS, BATOIDEI

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## PLATE 2 AND 21 TEXT FIGURES

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## INTRODUCTION

In an article in course of preparation, record will be made of dental abnormalities in the jaws of 9 recent sharks. In this paper the jaws of 21 species of rays will be studied.

In order to give the reader some idea as to what are the normal tooth structures in the rays under consideration, a brief statement is made for each family of rays studied. These facts are abstracted from Samuel Garman (1913), our foremost authority on the Elasmobranchii. For the rays studied herein I have used the names on the labels affixed to the dried jaws or applied by the authors quoted, without feeling the necessity of reducing these to synonymy. It is of course impossible even to assign anything more than the generic name when unlabelled jaws are studied, as will later be seen in three cases.

As in the case of the sharks, the question has come up whether to take up the abnormal teeth of fossil forms. But the difficulty is to determine what are the normal forms of such teeth. Finally it was decided to include only marked abnormalities in the fossil teeth of four extinct rays belonging to the family Myliobatidae, with the teeth of many of the modern forms with which I am well acquainted. These forms number only four; all the other rays studied are present day dwellers in our seas, and most of their abnormal tooth structures have never been figured or described before.

## DENTAL ABNORMALITIES IN RAYS, BATOIDEI

The jaws of rays are not such large and striking objects as are those of sharks and hence are less frequently preserved. Furthermore, their teeth are not so large and challenging as are the long slender tearing teeth of some sharks, and the broad triangular or sickle-shaped serrated chopping teeth of others. In fact in the small jaws of the rays the teeth are "in bands or pavements, compressed or cuspidate to broad and plate-like" (Garman, 1913, p. 257). They are, however, for some unknown reason, more subject to abnormal variations than are the teeth of sharks, as will be seen in the data presented in this paper.

A few specimens have been found in the collections of the American Museum, and more were found in the much larger collections of the U. S. National Museum which I have been privileged to study and describe. Descriptions of all these will, it is hoped, when combined with the widely scattered accounts in the literature, make an interesting section in this study of abnormal teeth in the elasmobranchs. It should be noted in this connection that a number of descriptions are of

previously published figures for which there are no descriptions in the accompanying texts.<sup>1</sup>

#### Family PRISTIDAE

The fishes of this family, which are really sharks on the way to becoming true rays, have the rostral cartilage produced and beset with teeth to form a saw—hence the common name, the sawfishes. The jaws, however, have extensive tooth-pads in which the roundish tile-like oral teeth are set in quincunxes.

#### *Pristis cuspidatus* (?), the Sawfish

In the collections of the American Museum is a pair of sawfish jaws taken in the Gulf of Aden and presented by the late Dr. Alfred Ehrenreich, who has sent in other like valuable material from this region to the American Museum. In these jaws the teeth are set in quincunxes and present a beautiful tessellate or pavement-like structure as if formed of squarish tiles. What the species is I cannot say positively. In the lower jaw the small tile-like teeth are in about 265–270 rows. The greatest number given by Garman (1913, p. 263) are 84–176 below for *Pristis cuspidatus* which is cosmopolitan for tropical and temperate seas. This presumably means for the whole breadth of the lower jaw.

Had the head, saw, and jaws come to us intact it would be possible to make a fair conjecture as to the species of this fish. All that can be said is that these jaws are nearer to those of *Pristis pectinatus* than to any fish listed by Garman. Furthermore this tooth-band and these teeth are, so far as I can determine by use of a magnifying glass, identical with those in the jaws of a *Pristis pectinatus* taken at Cape Lookout, N. C., and presented to me by the late Russell J. Coles.

In the left lower jaw, rather less than one-half the distance between the central part of the jaw (it has no apparent symphysis) and the outer left end, is the curious break in the continuity of the tile-like teeth

<sup>1</sup> The jaws of many of the Batoidei are composed of rather thin finger-like cartilages having the tooth-bands covering the outer, top, and inner surfaces. In order that the reader may be able to get a clear idea of the structure and arrangement of abnormal rows of teeth, segments of these toothbands are drawn as if the bands had been stripped from the jaw cartilages while "green," flattened, "cured," and drawn flat. Furthermore, especially when drawn in pairs, the upper is set in front of the lower with the two front or wearing edges brought close together. Drawn thus, right is right and left is left no matter which jaw is being discussed, and relative rows of teeth, upper and lower, are shown as they are in the mouth of the ray.



shown in Fig. 1. This figure has been drawn with the greatest care and accuracy, the teeth have been counted and every one checked. It is believed that it is the only figure in existence of a section of the jaw of a sawfish showing the oral teeth.

This abnormality consists primarily in the somewhat wide separation of the adjacent rows of teeth by a white line, especially in front where it is about 0.5 mm. wide. Note just here that in cleaning the teeth with a scrubbing brush preparatory to drawing and studying this jaw, a few teeth have been knocked out of the front. Backwardly the line is less wide, in fact on the rear there are two narrower lines with a central row of teeth. In front some of the teeth on the left of the line have their

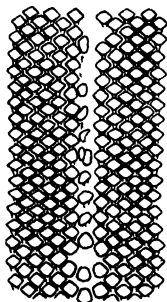


FIG. 1. A SECTION OF THE LEFT LOWER ORAL TOOTH-BAND OF THE SAWFISH, *PRISTIS CUSPIDATUS*, SHOWING A CURIOUS BREAK IN THE CONTINUITY OF THE TEETH

Note the flattening of the supernumerary teeth and the solitary row down the middle of the vacant space to the rear. Teeth  $\times$  about  $1\frac{1}{2}$  times. Specimen in American Museum.

sides next the line somewhat flattened. About on the top of the round part of the jaw there is a split tooth. Backwardly from this tooth run the two lines referred to above. The teeth behind this split tooth are rather widely scattered and almost entirely out of quincunx. The fourth of this row of teeth is large and placed in quincunx. Back of this is a large empty space looking as if a central tooth had been displaced, but examination of the jaw gives no evidence of such. Still further back are two large teeth so set as to fit accurately into the right and left oblique tooth-arrangements.

The question has already been asked by the reader "Is not this line an artifact caused by drying?" The answer is that it is not. Other such cases have been found, in jaws of other rays, but close study of

this specimen shows that it must be due to other and unknown causes. Note here the shape of some of these teeth, the interrupted quincunxes, and the split tooth. Nothing approaching this is found elsewhere in these tooth-pads—all is absolutely normal, with one slight exception. This is that at a few places in these jaws the teeth sometimes tend slightly to line up like soldiers on parade. This interferes slightly with the quincunx arrangement. On the jaws presented to me by Mr. Coles I find this somewhat more noticeable. But even on this latter this irregularity is so little pronounced that I myself never noticed it until I specifically looked for it. These minor matters are mere slight deviations from the normal and are in no wise comparable to that shown in Fig. 1. This arrangement in more exaggerated form will be shown later in the tooth-bands of *Raia stabuliforis*.

#### Family RHINOBATIDAE

The teeth of rays of this family are small, pavement-like, and set in quincunx. They are compressed or cuspidate to broad and plate-like. Those of the genus and species now to be considered are perhaps the most interesting and unusual of all the tooth-structures in any ray. Neither teeth nor jaw-structures for any member of the family have ever been described so far as I know.

#### *Rhynchobatus djiddensis*

In our collections is a beautiful pair of jaws without name or place of origin. Easily identified as *Rhynchobatus*, they must belong to the species assigned since the genus is monotypic (Garman, 1913, p. 268). Furthermore, since this species ranges from the Red Sea to the East Indies, it is concluded that these jaws must have been sent in from the Gulf of Aden by the late Dr. Ehrenreich.

Apart from the interesting form of these jaws and their pearl-like teeth of various sizes all arranged in quincunx, are the structures now to be described. In the deep central groove of the upper jaw are two definitely marked off lines running from front to back. That on the left (Fig. 2) separates larger teeth and these have large flat surfaces next the line. This line runs backward between three pairs of fairly large teeth and ends abruptly at a single large tooth which is irregular in shape and twice as broad as deep and hence is abnormal, as are the flat-sided teeth adjacent to and marking off the line. Finally in the next quincunx, behind the long tooth just mentioned, are two slightly abnormal teeth

instead of the one normal one due to be present. These are all shown in Fig. 2.

To the right of the center in the median groove of this same upper jaw is another well marked off line (Fig. 2). This line extends backward between 8 pairs of teeth flattened on their inner edges, and ends behind these against a tooth placed almost centrally to it. Back of this tooth where the line would have run, had it extended all the way, a number of teeth are placed and thus the line is lost. In this region the teeth are widely separated from each other and are somewhat irregular in shape, some rather markedly so. Finally the line again reappears though somewhat faintly, between the last lot of teeth—three on the left and two on the right, these themselves being somewhat abnormal. These of course do not show in the front view figure and it seems hardly necessary to make a drawing of the rear to show this very minor matter.

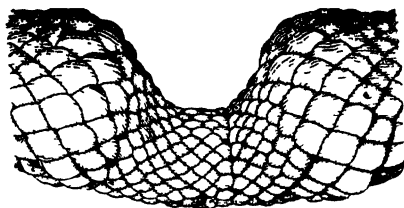


FIG 2 CENTER OF THE UPPER JAW OF *RHYNCHOBATUS DJIDDENSIS* SHOWING TWO LINES OF DIVIDED TEETH HAVING THEIR EDGES FLATTENED NEXT THE LINES

Teeth about half natural size Specimen in American Museum

One or two other cases of slightly flattened individual pairs of teeth occur on these jaws, but they form no such lines as have been figured and described. These lines are not the results of drying and cracking, but are true teratological structures as are the flattened teeth which make the lines plainly show. No cause can be assigned other than that these things arose in the tooth germs long ago. In process of time, as the back teeth would have come to the front by disappearance of the front teeth, these lined-up teeth would have disappeared. From this one must conclude that the disturbances in the tooth germs were temporary.

#### Family RAIIDAE

The members of this great family have the teeth small, numerous, and pavement-like—generally in quincunxes. In many forms the teeth

show a marked sexual dimorphism, as will be noted in the case of the first abnormality to be studied.

*Raia clavata*, the Thornback Ray

In the Raiidae but one case of tooth abnormality has, so far as I know, been hitherto described. Rays of this species, in keeping with the general statement set out above, show a marked sexual dimorphism in their teeth. The teeth of the males are pointed, while those of the females are blunt. Yet Parnell (1838, p. 438) affirms that he collected in the Firth of Forth three full-grown male specimens in which the teeth were as blunt as those of a female. Of one of these, a male two feet two inches long, he specifically writes:—"Teeth blunt, allowing the finger to be passed in either direction over their summits, without the vestige of a point being felt, the teeth being as blunt as those observed in the female species of the Thornback."

Day (Vol. II, 1884, p. 344, pl. CLXXI, figs. 1a and 1b) reiterates the statement as to the sexually dimorphic teeth and speaks of their "appearing much like the heads of large nails." He quotes Parnell and figures the teeth of *Raia clavata* but does not state in his text or elsewhere that they are drawn to illustrate Parnell's statement. However, his figure is labelled: "1a, teeth of female; 1b, teeth of male," hence we must conclude that they were drawn to illustrate this abnormality, this reversal of oral sex dimorphism. The matter is rather obscure, and as the drawings are very poor, it does not seem necessary to reproduce them herein.

*Raia stabuliforis* (*laevis*), the Barndoor Skate

In the collections of the U. S. National Museum are two pairs of unnamed jaws (No. 22234) of the same species. These were taken by Capt. J. W. Collins on the Grand Banks of Newfoundland. After study of the jaws and comparison with the teeth of a small specimen from Woods Hole in our collections they have been identified as belonging to the ray named.

One pair of these jaws shows certain irregularities in shape and arrangement of these teeth, set in quincunx, which will now be pointed out.

Between the eighth and ninth rows of teeth in the upper jaw, counting from the left, is seen a distinct line running from front to back. Under a magnifying glass it is seen that the teeth in the ninth row are normal in shape and size, but those on the eighth are flattened on their right sides, and this gives rise to the line referred to. In all these rows the

quincunx arrangement has disappeared. In the outer section of the lower right jaw, the teeth in the last eight rows are separated by lines from front to back. The line is most marked between those teeth which are the largest, most widely separated, and in the case of the front teeth most flattened. In these eight rows the quincunx arrangement is completely lost, the teeth standing in rows like columns of figures on a printed sheet very like those shown in Fig. 2. I can offer no explanation for this abnormality.

In another pair of jaws (U. S. N. M. No. 22234) belonging to this species, the lower jaw is entirely normal save for one of the familiar line-ups at the extreme left. The teeth are arranged practically everywhere in quincunx. However, about in the middle of the upper jaw—15

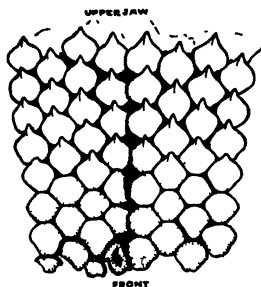


FIG. 3. A SECTION FROM NEAR THE CENTER OF THE UPPER JAW OF *RAIA STABULIFORIS*, SHOWING A LINE-UP OF TEETH ( $\times 1$ ) FROM FRONT TO BACK

Note the flattened edges in front and the overlapping behind. U. S. National Museum no. 22234.

rows of teeth from the right and 17 from the left—is found the condition shown in Fig. 3. This is a drawing made to show the tooth-band flattened out. The four anterior rows of teeth are flattened on their inner edges. The others overlap but stand opposite each other, i.e. side by side with the line between, and here again the arrangement in fives has disappeared to be replaced by twos or fours. Apparently the teeth, which would have made the center of each quincunx, have failed to develop. Here the teeth enlarged two times show this clearly. See Fig. 3 for details. The shading shows how the front teeth have been ground smooth by use.

There is still a third pair of jaws (U. S. N. M. No. 22235) of the barn-door skate to be figured and described, and this presents in the upper jaw greater abnormalities than any yet studied from this ray. Here

the lower jaw is comparatively normal save that at the outer ends of the tooth-band, especially at the ends, the small teeth have a tendency to line up in military fashion and hence the arrangement in fives becomes rather obscure. The teeth of the upper jaw are normal at the outer ends, and indeed everywhere save in the region shown in the drawing, Fig. 4. This upper right jaw has in drying suffered a recent break about midway between the symphysis and the outer end of the tooth-band. But to the left of this in the region of the abnormal teeth shown in the figure there is apparently an old and healed break of the jaw cartilage.

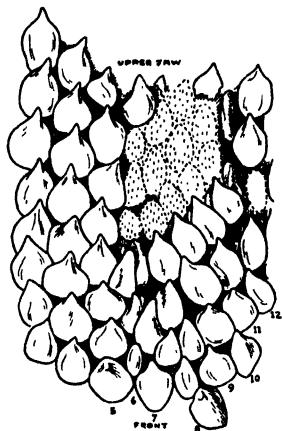


FIG. 4. SEGMENT FROM UPPER JAW OF *RAIA STABILIFORIS* ( $\times 1$ ) OF TOOTH-ROWS 6-12 TO THE RIGHT OF THE SYMPHYSIS

For details see the text. U. S. National Museum no. 22235.

Tooth rows 6-12 (counting to the right from the center) all show more or less deformation, crowding and displacement. Row 5 is entirely normal. Row 6 has but three teeth, increasing in size from front to back, but all undersized and malformed, the second especially so. Row 7 also contains but three teeth; the first nearly normal, the second pear-shaped, the third flattened and partly overlapped by tooth 3 of row 5. Row 8 has its first tooth about normal in size but displaced to the right. The second is small. The third has divided into two, while the fourth and fifth are about normal. Rows 9, 10, and 11 are more nearly normal in size but are crowded and out of quincunx. The teeth of row 12 are small—especially the two inner ones—and out of order.

The teeth of this jaw are more abnormal than the others of this species

previously considered, and indeed with one exception (presently to be studied) they are the most malformed of any ray teeth found in the course of this research. In the hinder part of this jaw the teeth in rows 6-11 are absent. Whether this absence is the result of the conjectured first break of the jaw cartilage or of the recent one due to drying cannot be positively decided, but the indications are that both are concerned. Probably this break due immediately to the contraction from drying was caused by a weakness in the jaw cartilages due to former fractures or hurts of some kind. There is on the inner side of the jaw cartilage at this point a thickening which points to a regeneration from an old hurt.

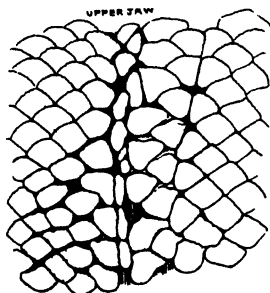


FIG. 5. MARKED ABNORMALITIES TO THE RIGHT OF THE CENTER OF THE UPPER JAW OF *DASYTIS HASTATA* FROM BEAUFORT, N. C.

For details see text. Teeth  $\times$  about 2. U. S. National Museum no. 27651.

#### Family DASYATIDAE

The rays of the sting-ray family have the teeth small, tessellate, and arranged in quincunxes. In all the tail is armed with one or more serrated spines as the common appellation of the various forms indicates.

#### *Dasyatis (Dasybatis) hastata*, the Stingaree

Radcliffe (1916) in his "Sharks and Rays of Beaufort, North Carolina," figures a jaw of a female of this species. He did not notice abnormal dentition in this, nor did I until after many repeated studies of his figure. The jaws in question have been lent me by the U. S. National Museum, in which they have been deposited. From the upper jaw Fig. 5 has been drawn.

The lower jaw of this stingaree is normal; the upper shows several

abnormalities—two visible in front view, and four in the rear (only two of which are shown in Fig. 5). It will be understood that the jaw plate is drawn flat (i.e., in a horizontal plane) as it would have been had it been stripped while "green" (uncured) from the jaw cartilage and flattened out.

In the extreme left of the upper jaw the first and second rows of teeth are abnormal in size, shape, and position. While between the third and fourth rows of teeth, counting from the right outer edge, is a straight line beginning at the back and running forward separating the teeth of these rows. Along this line, the teeth are out of quincunx and are more or less flattened on their inner edges to establish this line. The line is bordered by four pairs of teeth and in the region of a possible fifth pair is blocked by the corner of a tooth which stands obliquely in its way. In view of the greater abnormality now to be considered it does not seem necessary to figure these smaller departures from the normal.

Much more important are the great irregularities (Fig. 5) found to the right of the eighth row counting to the right from the symphysis of the jaw. Here is a much reduced row of teeth, smaller, flattened, and entirely out of quincunx, running clear from front to back. There are 11 of these teeth, all of different sizes and shapes. The fourth from the rear is especially abnormal—long and irregular. Notable is the fact that the teeth to the left of the abnormal row are all somewhat small and of a uniform size, while those to the right are much larger and are also markedly irregular in shape. In the drawing all teeth are enlarged about two times.

Between these lateral rows is a median row of very abnormal teeth. Numbers 1, 2, and 3 are about as long as normal but only about one-fourth the normal breadth. Number 4 has been segmented into two fragments. Numbers 5 and 6 are wider than the preceding but are abnormal in shape. So is number 7 which has been displaced to the right. Number 8 is in line, but is abnormal in size and shape. Since there are 9 recognizable distinct teeth on each side of the abnormal teeth, the last pair of median teeth may properly be interpreted as a divided ninth tooth.

The rear of this jaw presents an abnormality shown in this flat view but not visible when one looks at the jaw from the front in nature. Between the second and third rows of rear teeth, to the right of the major abnormality (Fig. 5) noted, is the familiar line extending forward plainly between three pairs of teeth and becoming obscured on top of the round of the jaw by reason of irregular placement of three pairs of



tooth-plate thus far found in this study, being composed of 12 rows of irregular teeth varying much in breadth. A careful translation of the author's description of the jaws has been made, but this is very lacking in detail. Though he calls this an anomalous specimen of the jaws, it is clear that he did not realize just how abnormal these are. A careful analysis follows.

There are 12 rows of hexagonal teeth, of which rows 1, 2, and 5 are of unusual breadth and form an ascending series in the order named. The fifth row has teeth of decreasing depth from front to back. In front (omitting the first and second rows of teeth) the breadth is three times the depth (from front to back), i.e., it takes three teeth to equal the breadth of the first tooth. The succeeding teeth grow steadily shallower, until at the rear it takes about four to equal the breadth of a

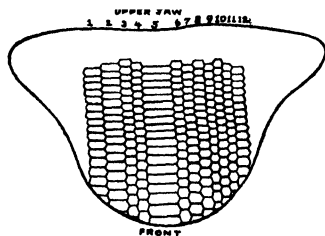


FIG. 7. THE UPPER JAW AND TOOTH-PLATE OF MYLIORATIS CHILENSIS (HALF THE SIZE OF THE AUTHOR'S DRAWING)

The details of this dental plate will be found in the text. After Philippi, 1892, pl. III, fig. 1-a.

tooth. There is even more irregularity in the relative front-to-back measurements of the teeth in rows 1 and 2. Generally speaking the teeth in any row are progressively thinner from front to back of the tooth plate.

The teeth of the ninth, tenth, and eleventh rows (counting from the left) are the most uniform in size. Those of rows 3, 4, 6, 7, 8, and 12 are also nearly of the same breadth. Use of the dividers brings out some interesting facts with regard to these rows. The teeth of row no. 5 are about three times broader than those of any of the right hand rows excluding no. 12. Thus rows 6-7-8 might have been formed by the segmentation of such a broad row of teeth. So also rows 7-8-9, 8-9-10, 9-10-11, and 10-11-12 might have been formed by the segmentation of such a row of broad teeth. Again the teeth of rows 3-4, 6-7, 7-8, 8-9, 9-10, 10-11 might have been formed by the break up of a row of teeth

having about the breadth of the teeth of row 2. Further, rows 9-10 and 10-11 are about equal in breadth to row one. Also rows 5-6 are nearly equal in breadth to rows 6-7-8-9 and to rows 7-8-9-10 on the right and to 2-3 on the left.

All of this is to say that these much segmented teeth are a great puzzle. There is no central row on which bilateral symmetry can be based. But it is significant that, taking the left hand ends of the teeth of the fifth and broadest row as a center, the tooth-plate is almost bilateral with the twelfth row as supernumerary (Fig. 7).

All the above points refer to the teeth of the upper jaw. Philippi does not figure the lower jaw of *M. chilensis*, and his description is very brief and inadequate. The lower tooth-plate was shorter and broader than the upper, and like it contained 12 rows of teeth of unequal breadth.

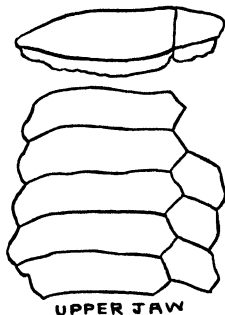


FIG. 8. FIGURE ( $\times \frac{1}{2}$ ) OF A FRAGMENT OF THE TOOTH PLATE OF *MYLIOBATIS DIXONI* FROM THE CRIMEA

Above, a central and a lateral tooth are shown in profile. After Obrutshev, 1928, p. 140.

The first, sixth, seventh, eighth, tenth, and eleventh rows were the narrowest and their teeth were slightly broader than long. There were no rows of broad teeth like those in the upper jaw. It is very unfortunate that Philippi did not figure the lower jaw.

Garman (1913, p. 430) describes and figures a new ray, *M. peruvianus*, without noting its habitat, from the collections of the Museum of Comparative Zoology (no. 636). In the section devoted to this ray he recognized *M. chilensis* as an abnormal specimen but was unable to allocate it to any definite species, accordingly it will simply have to go down in the literature under Philippi's designation but labelled abnormal.

There are now to be considered four species of fossil *Myliobatid* rays. Paleichthyology is a closed book to me, and while study of the figures of fossil elasmobranchs shows many wide variations, I shall confine myself to those with whose living forms I am fairly well acquainted, and from whose jaw-plates I can draw comparisons.

*Myliobatis dixonii* from the Eocene

After figuring and describing a number of normal jaws of this species, Dixon (1850) says in conclusion:

One singular example of part of the dental armour of the upper jaw of a species of *Myliobates* [*sic*], nearly allied to, or identical with the *M. dixonii* has the small lateral plates developed on one side only, the large or principal plates thinning off to an edge on the opposite side. This is doubtless an abnormal or accidental variety.

Dixon figures four jaws of this ray but in the above statement does not refer to a single one of these figures as representing this abnormality. Now the rays of the genus *Myliobatis* have pavement-like tooth-plates made of flat, hexagonal teeth in the form of a single median row of large (long) teeth and several lateral rows of smaller teeth on each side as has been shown in *M. californicus*. Hence Dixon's brief and indefinite statement lends itself to the conjecture that in his specimen the lateral teeth on one side were not preserved as were those on the other. However, this specimen was later purchased by the British Museum (Natural History) and is now in its collections. To it Woodward (1889, p. 110) refers in the following terms: "[No.] 25644. Abnormal specimen, having the lateral plates in the form of irregular parallelograms, referred to by Dixon, *op. cit.*, p. 198. Dixon Coll." One finds it difficult to question the statement of so eminent a paleichthyologist, but in the absence of a figure and more adequate description, it seems to me that this is a dubious abnormality.

We hear no more of abnormal dentition in *Myliobatis dixonii* until 1928 when Obrutshév figured and described a specimen of an upper dental plate from the Mnschankov limestone of Inkerman in the Crimea (Mus. Geol. Com., No. 2472). His figure is reproduced herein as No. 8. Of this specimen he says that large middle teeth occupy the place of the first and possibly of the second row of lateral teeth counting from the center out. On the right of this row of large teeth is a row of smaller hexagonal teeth.

Obrutshév's description is very brief and to me unsatisfactory. His

paper, except for three brief paragraphs, is mainly given up to a review of the literature and to a general discussion of the tooth-arrangement in Myliobatidae and Rhinopteridae. As in the case of the original *M. dixonii*, it seems to me that what this Russian scientist had is only a fragment of a jaw. I cannot accept it as a malformation in the absence of the other part of the jaw with which to make comparison.

*Myliobatis aquila* recent and from the Pleistocene

In 1905, Bassani published an article on the fossil Pleistocene fishes of Taranto and Nardo (Terra d'Otranto). Under the heading *M. aquila* he figures an abnormal upper jaw found in the Civic Museum of Natural History in Trieste. He quotes from a description by Maria Pasquale that of lateral teeth there are 27 on the left distributed in 3 rows and 20 on the right in 2 rows. His lithographed figure shows 20 on the right plus a fragment and 28 on the left with two others (outside ones) lacking,—one in the front row and another in the sixth row back.

The teeth of the outer row on the right have their outer edges flattened in normal outside fashion while those on the left have their outer edges rounded like the corresponding inner rows of teeth. Whether the author and artist thought that an outside row of teeth had been lost, cannot be said.

In his synonymy, Bassani does not quote Pasquale nor does he list any paper by her in his bibliography. The first article in this volume of the *Atti* is one by Pasquale on fossil selachians of meridional Italy. In this (apparently the first paleichthyological article ever published by her) other myliobatid teeth are described but there is no *M. aquila* nor synonym of it. Apparently this description quoted by Bassani was an oral or a holographic one.

Bassani's figure is not reproduced herein since his name for the ray has been reduced to synonymy and a better figure is given from Stefano in the next section.

Stefano (1914) states that among the specimens of *M. aquila* which he examined was a lower plate from a young living male in which the small teeth on the right side were in three rows and those on the left in two. He unfortunately does not figure this jaw in which the teeth are in reverse in comparison with those of Fig. 9 herein. Garman does not list either Bassani or Stefano in his synonymy of *M. aquila*, from which it may be inferred that he had not seen their papers. Neither does he refer to any abnormalities in the teeth of this ray.

In 1914 Stefano published an extensive memoir on *Myliobatids* fossil and recent. In his plates are figures of two abnormal lower jaws, which will now be considered. The first of these is synonymous with the species whose fossil tooth plate has just been described.

*Myliobatis bovina* from the Pleistocene of Taranto

On page 128, Stefano refers to the specimen just described from the Civic Museum of Natural History in Trieste by Bassani under the name *M. aquila*. He states that Bassani's specimen is really a *Myliobatis bovina* and hence he reduces it to synonymy. Then he adds that his figure 8, plate IV (Fig. 9 herein), is made from a photograph whereas

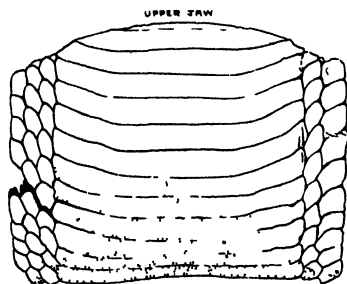


FIG. 9. AN ABNORMAL UPPER TOOTH-PLATE (HALF NATURAL SIZE) OF *MYLIOBATIS BOVINA* FROM THE PLEISTOCENE

Note three rows of small teeth on the left and two on the right. After Stefano, 1914, pl. IV, fig. 8

Bassani's figure of the same jaw was made from a drawing. Here are the 20 small teeth in two irregular rows on the right, with 28 in 3 rows on the left plus two missing. For the outer margins of the left outer row of teeth, the photograph corrects the drawing; the outer edges are flattened as is normal. Use of the dividers on these lateral teeth brings out the interesting fact that the 3 left rows are slightly wider than the two right ones—13 mm. to 12. Furthermore the right outer row of small teeth are the widest of all. What seems to have happened is that this outer right row has failed to divide into two, or an outer left normally single row has divided into two. Either supposition will, by virtue of the sutures between the small teeth, take care of the extra millimeter of width on the right. For all these points see Fig. 9.

*Myliobatis crassus* from the Pliocene

Stefano (1914, p. 143-146, pl. IV, fig. 12) figures a fossil lower dental plate of this species from the Pliocene of Orciano having a longitudinal groove down the center of the median teeth. In no other jaw nor in any figure or description known to me (save one presently to be referred to) of fossil or recent teeth have I found such a groove. For this see Fig. 10 herein. Stefano speaks of this furrow as being "quite wide and deep." Quite as abnormal is the irregularity in the sutures of the large median teeth in the bottom of this groove. If these things were found in a present day *Myliobatis* the teeth would certainly be pronounced abnormal. The V-shaped break in the front of the tooth plate

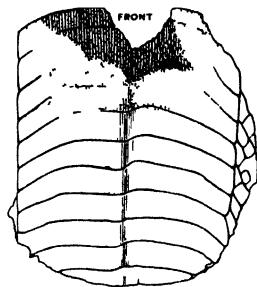


FIG. 10. LOWER JAW PLATE (HALF NATURAL SIZE) OF MYLIOBATUS CRASSUS FROM THE PLIOCENE

Note the groove in the center, the sinuosities of the median teeth in this region, and because of these the wide V-shaped break at the anterior edge. After Stefano, 1914, pl. iv, fig. 12.

is undoubtedly due to the crushing of mollusks just where depression in the teeth and twisting in the sutures met and made a line of weakness.

Stefano, at various points in his description of *M. crassus* (pp. 144, 145, 146, 147), speaks of other specimens (some undescribed hitherto) which show undulating sutures and very shallow grooves. On his pl. VI, fig. 5, he portrays a jaw showing these in far less development than in that reproduced as Fig. 10 herein. In both specimens, many of the lateral teeth are gone, but these are of no interest here as there is nothing to indicate any inequality in their rows.

## Family RHINOPTERIDAE

The rays of this family have the teeth angular, flat, broad, pavement-like, with a median series broadest of all. Flanking these are one or

more narrower rows of teeth but still broader than deep, and outside these two or more rows of small nearly equilateral hexagonal teeth. These teeth of varying sizes and shapes offer great possibilities for abnormal variations—especially in size. Accordingly relatively more cases of such abnormal dentition have been reported from members of this family than from any other.

According to Garman (1913, p. 443) in the genus *Rhinoptera* the "Teeth [are] tessellated, angular, in five or more rows, 5–19, median commonly wider." Furthermore, on a basis of tooth-structure Garman divides these rays into those having teeth in 7 rows above and below, and those having 9 rows in the upper jaw and 7 below; to these he adds two doubtful genera which, as will be shown later, are probably not distinct genera, but specimens with exceedingly abnormal teeth. Members of Garman's first group will now be taken up.

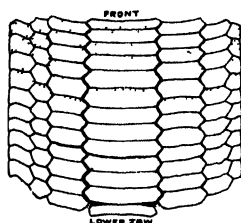


FIG. 11 LOWER TOOTH-PLATE (HALF NATURAL SIZE) FROM THE JAW OF A *RHINOPTERA QUADRILOBA* FROM BEAUFORT

Every left tooth row is of a different breadth from its correlative one on the right. Specimen in the author's collection

### *Rhinoptera quadriloba* (bonasus), the Cow-nosed Ray

This is the most abundant of the grinding-toothed rays found at Beaufort, N. C. During my 10 summers' work at the U. S. Bureau of Fisheries Laboratory there I must have handled at least a score of specimens. But unfortunately in those days I knew nothing of abnormalities and did not look for such. However, I did preserve two sets of jaws. One of these sets is perfectly normal above and below as is the upper jaw of the other, but the lower jaw of the second presents the interesting abnormality portrayed in Fig. 11.

Here there is a central row of 13 broad teeth (2 mm. broad by about 5 or 6 deep) flanked by three rows of narrower ones on each side, which show marked variations. To begin with, the central row of broad teeth is slightly out of center. The width of the three rows of right smaller

teeth is 24 mm., that of the three left rows is 22 mm. This condition is inherent and not due to the drying and shrinking of the teeth. However, the greatest irregularities are to be found in the relative widths of the corresponding rows of teeth. The teeth of the third and broadest left hand row measure about 12 mm. Those of the corresponding right hand row are 14 mm. broad. Similarly the teeth of the second left hand row measure 6 mm. in breadth while those on the right are about 10 mm. broad. Conversely, however, the teeth of the first and outermost left row give a measurement of 8 mm. (9 behind where the teeth are skewed); while those of the outermost row on the right measure 4.5 to 5 mm.

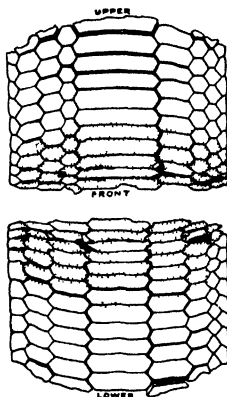


FIG. 12. TOOTH-PLATES (HALF NATURAL SIZE) OF A RHINOPTERA QUADRILOBA FROM BEAUFORT, N. C.

Note the very great unlikeness in the similar right and left rows of teeth in the upper jaw. The abnormalities in the lateral teeth of lower plate are not so pronounced, but are still quite marked. U. S. National Museum no. 27658.

(They are drawn too narrow by about the thickness of a line.) This jaw then is not only non-bilateral, but the correlative rows of teeth are each unlike its fellow.

Radcliffe, in his admirable paper (1916), incidentally figures the teeth shown in Fig. 12, but did not recognize that there were irregularities in both, nor did I until after long study of his figure. The specimen is in the U. S. National Museum (No. 27658) and has been loaned me for study. It has 11 rows above and 12 below, thus standing as an exception to the rule in Garman's listing noted above.

The upper jaw is bilaterally symmetrical, having in the center a row of 11 teeth about 25 mm. long by about 5 wide—certain narrower ones



will be noted later. On each side of the large central rows are four rows presenting marked abnormalities. On the right the first row from the center is composed of teeth 11 mm. broad, those of the next row measure 6 mm., those of the third, 5 mm., those of the outer, 4 mm. On the left the respective measurements are 5, 8, 9, and 4 mm. wide going successively to the left. Thus only the teeth of the outer row on each side are symmetrical with regard to each other and hence normal.

In the lower jaw is better symmetry. The 12 central teeth are 19 mm. wide by about 5 deep. Flanking these right and left is a row of teeth 12 mm. broad. Symmetry now breaks down, for on the right three rows of teeth equal the breadth of two on the left—about 12 mm. Thus the same tooth substance on the left, while equal to that on the right, has undergone less segmentation. Also it must be noted that on each side of the central row the lateral rows of teeth are thrust obliquely forward at their outer ends, somewhat like cusps. This is also somewhat noticeable in the upper jaw, but is more marked below.

Another abnormality worthy of attention is the shallower depth of the anterior teeth in both jaws. The front teeth are always more or less chipped from cracking mollusks and hence are not to be considered. The second tooth from the front of the upper jaw is also chipped, but a fair measurement for it is 3 mm. while the tooth behind measures 4 and the fourth 5 mm. In the lower jaw central teeth nos. 2 and 3 (from the front) are badly worn, and no. 4 hardly less so; and fair measurements for these give about 3 mm. But in contrast nos. 5, 6, and 7 measure 4.5 to 5 mm. deep. It is plain that in both jaws central teeth 2, 3, and 4 are less deep than the normal ones and their neighbors on each side partake to some extent of this same shallow depth. A similar abnormality near the center of the middle row from front to back will be noted presently in another pair of jaws.

Everywhere in this paper worn-down teeth are stippled, and as is shown in Fig. 12 the teeth of this ray have undergone great erosion by reason of its molluscan diet.

### *Rhinoptera lalandii*

Of the normal teeth in this species, Garman writes (1913, p. 445) thus—"Teeth in seven rows, in cases five in the upper jaw:—a median row of teeth three to six times as wide as long, a lateral row at each side of it in which the width is from two to three times the length [depth], and an outer two rows in which the width and length are about equal." In his text he makes no reference to abnormal dentition, but in his

plate 48 he portrays two sets of such teeth, one of which has 6 rows of teeth in each jaw instead of 7.

His figure 5 (Fig. 13 herein) is made from the "teeth of a medium sized specimen" (Mus. Comp. Zool. no. 534) which show asymmetry. The upper jaw of this specimen is bilaterally symmetrical (within the limits of good drawing) using the large median row of teeth as the center. These median teeth vary from 12 mm. breadth in front to 14 behind. The teeth of the left intermediate row are larger than those on the right, as are those of the left edge compared with similar ones on the right. To be more specific, tooth row 1 is wider than row 6 (counting from the left), and row 2 is wider than row 4, in fact it is nearly as wide as rows 4

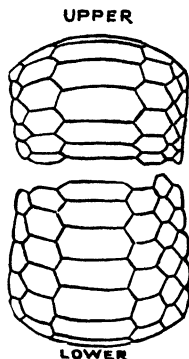


FIG. 13. UPPER AND LOWER JAWS OF A YOUNG RHINOPTERA LALANDII, WITH TWO ROWS OF TEETH IN THE LEFT SIDE AND THREE ON THE RIGHT OF EACH JAW

After Garman, 1913, pl. 48, fig. 5

and 5. However, there is the same amount of material in the two left and the three right rows of teeth.

The lower jaw, unlike the upper, is not only not bilaterally symmetrical with reference to the central row of broad teeth, but also is unsymmetrical in that it has two rows of teeth on the left and three on the right. The teeth of the central row vary in breadth from 11 mm. in front to 12.5 in the rear teeth. In depth they vary from 3-5 mm. The left lateral double row of small teeth measures 7 mm. wide, while the right three rows of like teeth average 8-9 mm. In short, the right side of this lower tooth-plate is somewhat wider than the left, using the central row of teeth as a center—7 versus 8 mm. The extra width is probably to be ac-

counted for by the sutures formed in the segmentation of the extra right row of teeth.

Fig. 6 on Garman's plate 48 was drawn from "a large specimen of *R. lalandii* (Mus. Compar. Zool. No. 534)." The upper jaw is bilateral—the central row of long teeth being truly median. All the measurements of all the teeth check up within the limits of good drawing. This does not refer merely to the widths of the right and left rows of teeth taken together, but also is true for the teeth of the similar rows. The widths of the teeth in the second-sized right and left rows vary slightly, but this may be due to slight inaccuracies in the drawing. This jaw is then entirely normal and hence is not figured.

Turning to the lower jaw (Fig. 14), it is found to consist of a central row of broad teeth (measuring in the figure 12 mm. broad by about 5

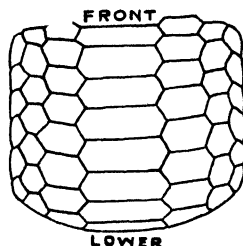


FIG. 14. LOWER JAW ONLY OF A LARGE RHINOPTERA LALANDII (M. C. Z. NO. 534)

While each side of the tooth-plate has three rows of small teeth, the plate is still unsymmetrical. After Garman, 1913, pl. 48, fig. 6.

deep) with two sets of three rows of teeth right and left. However, the dividers show that this tooth-plate is not symmetrical. From the left extremities of the broad central teeth to the left edge the measurement is about 10 mm. while on the right it is 12 mm. The reason for this seems to be found in rows 3 and 5. Rows 1 and 7, and 2 and 6 are very closely of a size (breadth), but row 5 is about 1.5 mm. wider than row 3 (always counting from the left). The other half millimeter is found in almost infinitesimal differences in the right hand rows of small teeth.

#### *Rhinoptera marginata*

Of this pavement-toothed ray, Garman (1914, p. 445) says, "teeth in nine rows; median row nearly three times as wide as long," etc. His figure (pl. 48, fig. 4) is labelled "Jaws and primary dentition." These jaws show various transition stages which are chargeable to immaturity

and it does not seem necessary to reproduce his figure. However, Owen (1840, I, p. 47, fig. 2, pl. 25) after describing the normal dentition of the genus *Rhinoptera* says of "*Myliobates marginata* (subg. *Rhinoptera* of Kuhl)" that "I have seen an example of the jaws of this subgenus of *Myliobates*, in which one of the rows next the median was subdivided into unequal series as represented in fig. 2, pl. 25." His figure is reproduced herein as Fig. 15. As may be seen, Owen's figure represents not the whole tooth-plate but merely a section out of what may be presumed to be the middle region.

This tooth plate under the dividers is symmetrical measured from the extremities of the broad median teeth. Row one on the right is equal in breadth to the first two rows on the left. The teeth of the second row on the right and those of the third on the left are equal in breadth within the limits of good drawing. Right row three has teeth of the same breadth as left row four. Owen did not remark on the up-and-down

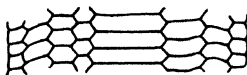


FIG. 15. A SEGMENT OF AN UPPER JAW OF RHINOPTERA MARGINATA SHOWING ABNORMAL DENTITION IN THE FIRST AND SECOND ROWS OF TEETH TO THE LEFT OF THE CENTRAL ROW

Author's figure  $\times \frac{1}{4}$ . After Owen, 1840, pl. 25, fig. 2

shifts in the axes of the teeth of lateral right rows two and three, nor on the diagonal downward slant of the teeth of lateral rows three and four on the left, but attention should be called to these irregularities. However, the chief abnormality here consists in the fact that the left row of a secondary broad set of teeth has been broken into two rows of teeth of tertiary size.

In his text and in his descriptions of his figures Owen designates this figure by the names set out in the first paragraph, but on his plate he labels it "*Zygobatis*." Bateson (1894, p. 260-261) reproduces this figure with right tooth row two and left three wrongly drawn (slanting in directions opposite to those found in Owen's figure) and labelled *Rhinoptera javanica*—on what authority I do not know. But it should be stated that *R. marginata* and *R. javanica* are quite similar to each other and hence near in the system. Bateson notes that this is a Hunterian specimen in the Royal College of Surgeons.

*Rhinoptera jussieu*

According to Garman (1913, p. 447) this ray, whose habitat is Brazil, has "teeth in 9 rows (7-10);—three median of wide teeth, next outward in most cases about as wide as long, and in the outermost one, two, or three rows the width equals the length." Then he adds "The variability of individuals of this species is excessive"—a matter which will now be demonstrated.

In 1888, Woodward figured and described an interesting case of abnormal dentition in a ray. This he identified as the Brazilian species above and thought that the jaw came from an adult. This is shown herein as Fig. 16. In it we must conclude that *Oa* is the central row of teeth and that the right hand rows of teeth marked I, II, III, IV are normal in size and shape and hence that the abnormality is to be found in the left side of the jaw. Here the tooth rows II, III, and IV are very

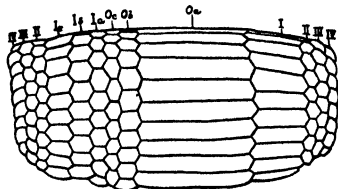


FIG. 16. AN EXCEEDINGLY ABNORMAL TOOTH-PLATE (NOT DESIGNATED AS UPPER OR LOWER) OF RHINOPTERA JUSSIEUI FROM BRAZIL

Original figure reduced one-half. After Woodward, 1888, p. 282

similar in shape and appearance to those on the right, but collectively they are wider by 1 to 2 mm. due to the fact that left IV is wider than right IV by just this measurement. However, they are evidently homologous with these. The abnormality there is to be found in the fifth tooth rows *Ob* to *Ic*. Of these the rows *Ia*, *Ib*, *Ic* seem plainly to be the homologue of I. The measurements correspond very closely, *Ia*, *Ib*, and *Ic* running 1-2 mm. longer than I. This slightly greater width may possibly be accounted for by reason of the two extra sutures on either side of *Ib*; *Ob* and *Oc* would then seem to be the interpolated teeth, formed by the subdivision of an extra tooth row corresponding to but shorter than I.

I cannot find a point of bilaterality in this jaw. Woodward suggests that this can be found by presuming that *Ob* and *Oc* have been broken off *Oa*. But if one using dividers measures right I, II, III, and IV, the same measures on the left include IV, III, II, *Ic* and *Ib*, and *Ia* is left

unaccounted for. Woodward's supposition then might hold, if tooth-row *Ia* is considered an accessory row of teeth.

Another interpretation is to consider *Ib* and *Ic* as the homologue of *I*, although combined these teeth are about two to three millimeters shorter than *I*. They certainly look to be the segmentation products of a band of teeth like *I*. On the above supposition tooth rows *Ob*, *Oc*, and *Ia* are interpolated teeth, probably the products of an extra row of teeth like *I*, but less broad.

However, there is still another way to look at the matter and this is to consider *Ob* and *Oc* and *Ia*, as the homologues of *I*. Here the left three run slightly smaller than *I*, about 2 mm. on the average. Then *Ib* and *Ic* must be considered as the interpolated teeth, the products of an extra long row like *I*. However, the former interpretation is possibly the better since the teeth marked *Ib* and *Ic* are plainly the products of the segmentation of elongated teeth like *I*. In any case the bilateral symmetry of this jaw is obscured by the absence on the left of tooth row *I*, and the presence of the tooth rows *Ob* to *Ic* inclusive.

Woodward, however, puts forth another explanation as follows:

As the large mesial teeth are decidedly unsymmetrical and do not quite occupy the middle part of the dentition, their extremities on the abnormal side also seem to have been detached. Indeed it will be noticed that if the first two of the abnormal lateral rows (*Ob*, *Oc*) could be connected with the very broad teeth, the latter would [not] be precisely median; and the manner in which the length of the teeth of the second of these series varies with the differences in the length of the broad teeth seems to prove that the homology denoted by the lettering is correct. The three rows marked *Ia*, *Ib*, *Ic* taken together are [not] exactly equal in breadth to the first row of the opposite side, and may be regarded as its equivalent.

Bateson (1894, p. 259) adopts with reservations Woodward's idea that the plates of the row *Ob* have arisen by division partly of the left end of the central plate and partly from lateral row *I*. But he thinks that these supernumerary teeth have undergone a rearrangement also. However, neither Woodward nor Bateson has applied dividers and has measured the tooth-bands. If *Ob* and *Oc* are added to the broad teeth *Oa*, the combination would not become median. From the right extremities of the tooth row *Oa* to the outer edge of the jaw, the distance varies from 22 to 25 mm.; while from the outer points of the teeth marked *Oc*, the distances vary from 26 to 28 mm. I do not believe that any teeth have been divided off the left extremities of the teeth in row *Oa*.

That Woodward is incorrect, as I have indicated by the bracketed word [not] inserted in two places, the reader can ascertain by the use of dividers. That Bateson is also incorrect may be ascertained by the same simple means. The drawing, made from a tracing of Woodward's original figure, has been checked with dividers and found to be minutely correct. No point of bilaterality can be found and the homologies of these various rows of abnormal teeth in this, the most abnormal tooth plate yet studied, must be left an unsolved puzzle.

In his plate 48 (figure 3) Garman (1913) portrays abnormal dentition in a large specimen of this ray (Museum of Comparative Zoology, No. 535). Of this ray he merely notes (text, p. 447) that "The variability in

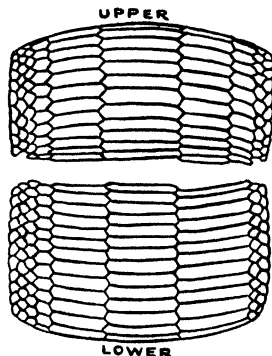


FIG. 17. ABNORMAL UPPER AND LOWER TOOTH-PLATES FROM A LARGE SPECIMEN OF RHINOPTERA JUSSIEUI IN THE MUSEUM OF COMPARATIVE ZOOLOGY (No. 535)

For details, see text. After Garman, 1913, pl. 48, fig. 3

individuals of this species is excessive." He does not describe this teratological specimen. The teeth are in nine rows normally, three rows consisting of wide teeth, and this is a matter of specificity. In the figure (No. 17 herein) there are ten rows in the upper jaw; three rows of broad teeth in the center, four of narrower ones on the left and three on the right.

The broadest central row of teeth in the upper jaw is bilaterally placed as may be found by using dividers. The right and left broad teeth are practically of the same breadth—within 0.5 mm., the limits of good drawing. The same holds good for the group of outlying smaller teeth on each end of the jaw. Since the outermost teeth right and left are

equal in size, it follows that the three inner smaller rows on the left and the two inner smaller rows on the right must be equal in width and the dividers so show. Hence it is proved that the tooth-material that made two rows of small teeth on the right has been divided into three rows on the left, thus keeping the tooth-plate bilateral. But this is not the only abnormality. The two outer rows left and right are approximately of equal breadth. Then it follows that teeth of left rows 3 and 4 must equal the breadth of those in row 8 on the right and the dividers show that this is so. Hence the prime factor in the abnormality of this jaw lies in the fact that a left row of tertiary breadth corresponding to row 8 on the right has been segmented into two rows of smaller teeth.

In the lower jaw there are 9 rows of teeth in contrast with 10 above as may be seen in Fig. 17. There are three central rows of oblong teeth of which the teeth of the first and second correspond in breadth with the like in the upper jaw. This lower jaw is bilateral with respect to this second broad central row of teeth. On the left are four rows of small hexagonal teeth identical in number of rows and in total breadth with the similar four in the upper jaw. On the right of this lower jaw are two rows of small teeth homologous in number, size, and shape with the like two rows above.

There are two abnormalities in this lower jaw. The patent one is that on the right for some reason there has failed to segment off from the third broad central row of teeth a row of secondary teeth corresponding to row 8 on the right of the upper jaw. The dividers show that the breadth of the teeth in upper right rows 7 and 8 exactly equals the breadth of the teeth in lower right row 8. The second abnormality is that on the left there is a row of teeth, no. 4, broader than the teeth in rows 1-2-3. Furthermore, it is interesting to note that the total breadth of the teeth of rows 4-5 on the left is exactly equal to the breadth of the teeth of row 8 on the right.

#### *Rhinoptera polyodon*, the Multi-toothed Rhinoptera

In his "Catalogue" (1870, VIII, p. 495), Gunther lists this ray from a pair of jaws (my Fig. 18) of unknown source. Here follows his description—"Upper jaw with fifteen series of hexangular teeth, those of the five middle series being but little broader than those of the outer ones. Lower jaw with nineteen series of similar teeth, those of the five middle and of the outer series being nearly twice as broad as the others "

Let us examine these teeth somewhat critically. In the upper jaw there is complete symmetry, so far as the eye can discern, with the eighth



row in the center. But there is not complete symmetry in the individual rows. Left rows 6 and 7, and right rows 9, 10, and 11 are practically of the same breadth as row 8. There are then six central rows of teeth of approximately equal breadth, not five as Günther states. There are slight irregularities in some of the other rows but they are too insignificant to have attention called to them.

This symmetry, however, does not hold for the lower jaw. This is practically bilaterally symmetrical if one uses dividers but not if one counts rows of teeth. Having fixed the central row of teeth, it will be

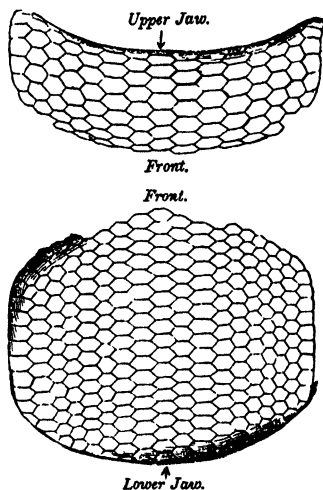


FIG. 18. TOOTH-PLATES OF THE MULTI-TOOTHED RHINOPTERA (*R. POLYODON*)

These are the most abnormal tooth-plates ever figured and described. After Günther, 1870, vol VIII, p. 495.

found that on the left side there are 8 rows of teeth whereas on the right there are 9. This gives 18 rows of teeth ( $8 + 1 + 9$ ) instead of 19. Nineteen rows are found in transverse count only when one reaches the point on the upper left where the outer row has become divided into two rows. The count is one greater on the upper left because here there are three rows of small teeth instead of two rows of larger ones. The outer row has divided into two and the anterior teeth of old row two have decreased in size. However, the breadth of the three new rows of small teeth is the same as that of the two old broader rows. Other small irregularities, too insignificant to be considered, may be seen on

close inspection. Plainly this is an abnormal *Rhinoptera* jaw with the broad plates many times subdivided.

In 1880, Günther in his "Introduction to the Study of Fishes," says of this ray that "Nothing is known [of it] except the jaws; and as its dentition is very peculiar, no opportunity should be lost in obtaining and preserving entire animals." However, he seems never to have had another specimen; nor has one ever been taken. Garman (1913, p. 448) lists this fish with Günther as his authority for this sole specimen. However, a specimen similar to this will now be referred to.

### *Rhinoptera encenadae*

In 1886, Rosa Smith (Mrs. C. H. Eigenmann) described a jaw of hexagonal teeth which could only have come from a *Rhinoptera*. This jaw was found on the beach at Encenada, Lower California. The plate was much worn, probably by the weather as well as by use, and had lost some teeth from both front and back edges. It was sent to the U. S. National Museum, in the *Proceedings* of which the description was published. This reads as follows (Smith, 1886):

It is a lower jaw and has fourteen series of hexangular teeth, the sinistral outer row nearly twice as broad as the narrowest inner series, the dextral outer series a little broader, just equaling two of the narrowest series and one and a half times broader than long. The teeth of the two sides of the jaw differ from each other. Inside the sinistral outer series are four rows of smaller teeth, as broad as long, nearly uniform in shape and size, the width of the four rows collectively equaling one and two-fifths times the width of the series of the broad teeth just inside them; the teeth in this row are the broadest of the jaw and about twice as broad as long. The seventh sinistral series has teeth that are somewhat enlarged, two-thirds the diameter of the sixth and widest series just described, scarcely broader than long. Inside the dextral outer row are two rows of enlarged teeth, slightly larger than the seventh sinistral series; immediately inside these are three rows of narrow teeth, similar to the four rows of small teeth next the sinistral marginal row; the seventh dextral row and the central series contain small teeth that coalesce into a single row a little back of the center of the jaw. The jaw anteriorly has fifteen series of teeth, and posteriorly only fourteen, by the coalescence of the teeth of the central and seventh dextral series, as above stated.

It is most unfortunate that no figure was made and without such the description is almost wholly unintelligible. But the jaw most surely belonged to a *Rhinoptera* and as surely the one to which it is most nearly related is Günther's *R. polyodon*. However, it differs from this

in being somewhat unsymmetrical, in that the sixth row of teeth from one side (eighth from the other) is the broadest, and also in having only fourteen rows of teeth instead of nineteen. Miss Smith concluded that it was a new species and named it as above. Garman so lists it in his work but says, "Named from an abnormal lower jaw with fourteen rows of teeth unlike on the two sides."

This specimen has disintegrated into the individual teeth and these are preserved in a bottle in the U. S. National Museum (No. 37966) where I studied them. There are 180 of these separate teeth which if arranged in 14 rows would give about 13 teeth to the row. With the figure of *Rhinoptera polyodon* before me I was able to pick out of these 180 teeth: (1) 13 of the largest size double-ended teeth which were 8-9 mm. broad by 4-5 deep; (2) 27 of the second size, 6-7 mm. broad by 4-5 deep; (3) 21 marginal plates with one edge smooth and rounded, measuring 7-9 mm. broad by 4-5 deep; (4) the remainder of much smaller teeth which I could not classify. From these one can reconstruct one row of broadest teeth, one and one-half of the second sized teeth, and almost the two marginal series of teeth. The broad teeth are almost identical with the like ones in *R. polyodon*, the smaller teeth are remarkably similar to its smaller ones, and the marginal teeth are identical.

One hardly knows what to make of these jaws of *Rhinoptera polyodon* and *R. encenadae*. They almost certainly belong to the genus *Rhinoptera*, and they as certainly are abnormal. The single fact, that no other specimens of these rays have come to light in the 63 years in one case and the 47 in the other since their discovery, while very significant, cannot absolutely be taken to mean that these jaws and teeth may not represent valid species. However, the soundest conclusion seems to be that these jaws are abnormal comparable to that of *R. chilensis* figured from the fish itself, and as such they are described herein.

In this connection, in so far as Günther's *R. polyodon* is concerned (and by inference Smith's *R. encenadae* also), I am glad to find my conclusions are in accordance with those of so eminent a student of variations and abnormalities as Bateson (1894, pp. 259-260), who in speaking of Günther's set of jaws writes, "This specimen is described as *Rhinoptera polyodon*, but it is by no means unlikely that it is actually a variation derived from the usual formula of *Rhinoptera*." These remarks apply equally well to *R. encenadae*.

*Rhinoptera* sp (No 1)

There is in the collections of the American Museum a pair of *Rhinoptera* jaws without specific name or place of origin. These tooth-plates present some interesting abnormalities which will now be considered. These are shown in Fig 19 drawn as in a flat plane.

The upper jaw is bilaterally symmetrical in regard to its central row of teeth and in all other respects. The 10 teeth (one broken off) of the median row are 14 mm broad. Those of the first left and right lateral

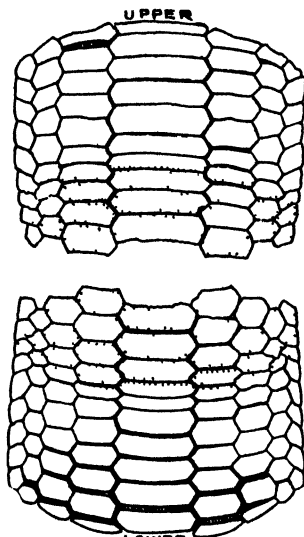


FIG 19 UPPER AND LOWER JAWS (NATURAL SIZE) OF A RHINOPTERA OF UNKNOWN SPECIES HAVING THE FOURTH AND FIFTH TEETH OF EACH CENTRAL ROW ABNORMALLY SHALLOW IN DEPTH AS ARE THEIR NEIGHBORS TO THE LEFT AND RIGHT

Specimen in American Museum

rows are of the same breadth—8 mm. The teeth of the two outer rows are of the same breadth for each row and together measure 8 mm.

The lower jaw is also bilaterally symmetrical with relation to the large central teeth which are 10 in number, and are 11 mm broad. The first lateral rows are identical in size—7 mm broad. So are the second lateral rows on each side measuring 6 mm broad. But the third right lateral row is broader than the corresponding left row, and on the other hand the left marginal row is wider than the right marginal one. How-

ever, the last two right laterals and the last two left laterals are equal—making the whole jaw bilateral.

To the eye and under the dividers the two tooth-plates are bilaterally symmetrical—the one small variation noted not interfering. However, there is one marked abnormality in each jaw. In the upper jaw the central teeth are 4 mm. deep on the average, but number 4 from the front is but 2.5 mm. deep and no. 5 measures 3 mm. In the lower jaw, the large front median teeth are 4 mm. deep, while nos. 4 and 5 are but 2 mm. deep. Median teeth nos. 7–9 are but 3 mm. deep. This, however, is explainable in that these latter were young and non-hardened, and in drying they have lost a millimeter in depth.

These abnormally shallow teeth have those adjoining them also decreased in depth. This is noticeable in the upper jaw but in the lower is very marked. Such abnormalities do not seem to have been noted before. For all these things see Fig. 19.

As to the identity of this specimen, I am at an utter loss. In his key, Garman (1913, p. 444) makes his second group of Rhinopterid rays have 9 teeth in upper jaw and 7 in lower. This specimen, as Fig. 19 shows, has 7 above and 9 below. If Garman has possibly inverted his figures then the ray belongs in his second group. Then on the basis of tooth measurements these jaws seem to qualify for *R. marginata*, but it will not do to designate a species merely on the basis of tooth counts and measurements.

### *Rhinoptera* sp. (No. 2)

In the collections of the U. S. National Museum there is a set of *Rhinoptera* jaws (No. 27672) with 7 rows of teeth in the upper jaw and 8 in the lower. The lower jaw is bilaterally symmetrical and the teeth normal under the dividers row by row. However, the upper jaw (Fig. 20) has an extra row of teeth. The 11 teeth of the broad central row are 18 mm. broad by 4 deep. Those in the rear are slightly deeper because as yet unhardened, those in front are slightly narrower because they are worn down considerably—the teeth are deepest or widest at the crown and narrower toward the base. The jaw is slightly unsymmetrical—the three rows of teeth on the left side are 13 mm. wide; the four on the right measure 15 mm. The two long rows right and left each have teeth 8 mm. broad. The teeth of the next outer row are of the same breadth (5 mm.) within a small fraction of a millimeter. The teeth of the left outer row are about 0.5 mm. wider than those of the right outer. From these measurements it is evident that the teeth of

the second row from the right are interpolated, that they are the teeth which make this jaw unsymmetrical on the right. All these matters are clearly illustrated in the drawing—Fig. 20.

*Rhinoptera* sp. (No. 3)

Bateson (1864, p. 260, fig. 69) portrays and describes a section of an abnormal Rhinopterid jaw from the Museum of the College of Surgeons (Hunterian specimen). This figure, which is reproduced herein as

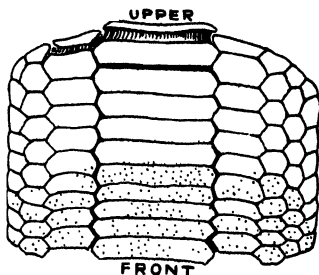


FIG. 20. UPPER JAW (NATURAL SIZE) RHINOPTERA SP. NO. 2 HAVING THREE ROWS OF SMALL TEETH ON THE LEFT AND FOUR ON THE RIGHT

U. S. National Museum no. 27672

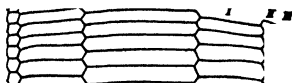


FIG. 21. SECTION OF A JAW (WHICH NOT SPECIFIED) OF AN UNKNOWN RHINOPTERA (NO. 3. HEREIN) SHOWING BEGINNING SEGMENTATION IN THE SECOND RIGHT HAND ROW OF TEETH

Original figure reduced  $\frac{1}{2}$ . After Bateson, 1894, p. 260

Fig. 21, shows the smallest abnormality yet described. The jaw is entirely symmetrical save for the fact that the right hand row of small lateral teeth marked II has not undergone segmentation as has the row on the left. Three of these teeth have begun each to break into two, but the lower three show no signs of division. The dividers show rows I and I right and left to be of the same breadth. The unsegmented II on the right and the segmented II on the left are of the same breadth. I will not hazard a guess as to the identity of this specimen.

## Family MOBULIDAE

These fishes are of considerable and some of very large size, and all are of rather restricted tropical and subtropical distribution. Little is known about their dental apparatuses. One paper (Pellegrin, 1912) has been written dealing in a general way with the dentition of fishes of this family, and particularly of the form next to be considered. Pellegrin had but two specimens of this ray and studied the teeth mainly from the viewpoint of classification and of sexual dimorphism in the structure of the teeth of the species. He had no opportunity to see any dental abnormalities.

*Mobula hypostoma (olfersi)*, the Small Devilfish

Radcliffe (1916), in the excellent paper elsewhere referred to, has figured the tooth-bands of a female fish 1420 mm. (55.9 in.) long taken at Cape Lookout, North Carolina. He noted no abnormalities in the dentition nor did I until after repeated examination of the jaws themselves. The specimens are all in the collections of the U. S. National Museum, and, with other valuable material noted above, have been loaned me for study and photography.

In Figs. 1 and 2, pl. 2, are shown the teeth of a male of this species, the upper teeth (Fig. 1) above, the lower below (Fig. 2). In trying to point out variations in these teeth one must first try to determine the normal. Studying the upper tooth-plate of the male (Fig. 1, pl. 2) as a whole, it would seem that these broad-based overlapping teeth have normally one, two, or three points or cusps. Teeth with two cusps are in the majority and these are probably the normal teeth. Studying the teeth under a glass, it would seem that those with single points are the ones which have failed to develop fully. These are found in rows in which the majority of the teeth have two cusps. Occasionally one with three points is found in such a row. All these can probably be called mere variations. At the very ends of the tooth-band right and left are found teeth of very unusual form having two, three, and four cusps. These teeth are so out of the ordinary, so unlike any of the others, that they probably may be properly classed as abnormalities. Then near the center of the tooth-band is a row of teeth having (counting from the front) three rows of teeth with two cusps, two with three cusps, two with four cusps, and one with five points. Surely this row of teeth can be called abnormal.

Possibly this row is a symphysial row of teeth (and such are always

unlike the others) but from the center of the rear tooth of this row to the left edge of the tooth-band is 30 mm., to the corresponding point on the right 32 mm. However, the facts are that this jaw is unsymmetrical and that it has this row of very unusual teeth near the center.

Let us now turn to the lower tooth-band of the male, in which will be found many striking abnormalities (Fig. 2, pl. 2). The normal teeth are like those in the upper jaw, i.e., each has a broad base and on this a tooth with two backwardly projecting cusps. The abnormalities are many and interesting. The teeth of the second row from the left end of the tooth-row have three cusps. So have the inner teeth of row 4 and the outer of row 5. Row 13 has three cusps to a tooth. The teeth of row 14 have four and five and six cusps. Row 18 has multicusped teeth—some with as many as six cusps. The teeth of row 30 are also most unusual of all, the cusps running four, five, and six—some of the latter being very uniform. Still more abnormal is tooth row no. 36. Its teeth have five to six well developed main cusps and two smaller ones on their outer edges. Tooth rows 45 and 46 (nos. two and three counting from the right) are likewise abnormal. They are twice as broad as normal teeth and carry varying numbers of cusps. All in all this tooth band is one of the most abnormal found in this study.

In the female *Mobula* the teeth (Figs. 3 and 4, pl. 2) are broad and flat (entirely lacking in points) and are definitely arranged in quincunx. Here the abnormalities consist of broad undivided tooth-rows. In the tooth-plate under consideration (the upper—Fig. 3, pl. 2), the first and second and third rows of teeth (counting from the left) are very much wider than the normal ones. The twenty-eighth row from the left (the twenty-sixth from the right) is of double breadth. Apparently these teeth have arisen from an unusually large tooth-bud—perhaps from one which has failed to divide or from a pair of coalesced tooth-buds. The anterior tooth and also the last but one from the rear have divided into two teeth, about normal in size but with their inner edges not rounded. Rows 35 and 36 are also of unusual breadth. At the extreme right a number of rows (particularly the last two) are, like those at the extreme left, of unusual breadth.

In the lower jaw of the female (Fig. 4, pl. 2) a very similar state of affairs is found. However, it is noticeable first of all that the teeth in the various rows are set more widely apart than those in the upper jaw—there is very little overlapping. This wide separation tends to break up the close quincunx formation. There is some variation in the breadth of the teeth—especially noticeable in the anterior teeth of row 17. Very



broad are the teeth of rows 21, 22, and 23. However, broadest of all are the teeth of row 35, in which the teeth are nearly double the normal size. Radcliffe (1916, p. 279) notes the greater breadth of these tooth rows and of those in the upper jaw, but did not realize that they were abnormal. I also did not recognize this until I made a special examination of the teeth themselves for such malformations.

From these figures it is plain that in *Mobula hypostoma* there is not merely a very unusual sex dimorphism in the teeth of males and females, but that there are very great abnormalities in the teeth of each sex.

#### SUMMARY

Of the 21 rays whose jaws and teeth are studied herein, abnormal dentition had been definitely recorded as such in the literature for but two, one fossil and one recent species. Herein are given twelve descriptions from the literature of abnormal dentition in eleven rays (two descriptions for one ray and an old and a new for another), illustrated by eight figures. These descriptions and figures (other than for the two cited by title in the bibliography) are but briefly referred to in the literature and then mainly incidentally (generally in the systematic literature). Sex dimorphism is noted in the teeth of two rays.

For eleven rays (one counted in both old and new) descriptions are given for the first time of 15 sets of malformed teeth. Descriptions of three lots of teeth are set out for one species and two each for two other species; and in a third form for both male and female rays; a total of 18 new descriptions. These accounts are illustrated by 17 figures, of which three are copied from Garman (who did not recognize them as portraying abnormal teeth), and ten are drawn and four photographed from the jaws themselves. These 14 are figured for the first time for their abnormal teeth. If there are teratological teeth in both jaws, both are shown in one figure, save for *Mobula olfersi* where upper and lower jaws are shown separately for each sex (the sex dimorphism here being very marked). The greatest number of specimens of malformed teeth were found in the rays of the families Myliobatidae (7) and Rhinopteridae (12).

Rays of 21 species were studied with a total of 30 jaws in which abnormal dentition was found and described in upper or lower tooth-plates and not infrequently in both. In one specimen only was the possible cause found in an old fracture in the jaw cartilage. Of each of the other 29 specimens, all that can be said is that the abnormalities were produced by unknown causes internal in the tooth germs.

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## PLATE 2

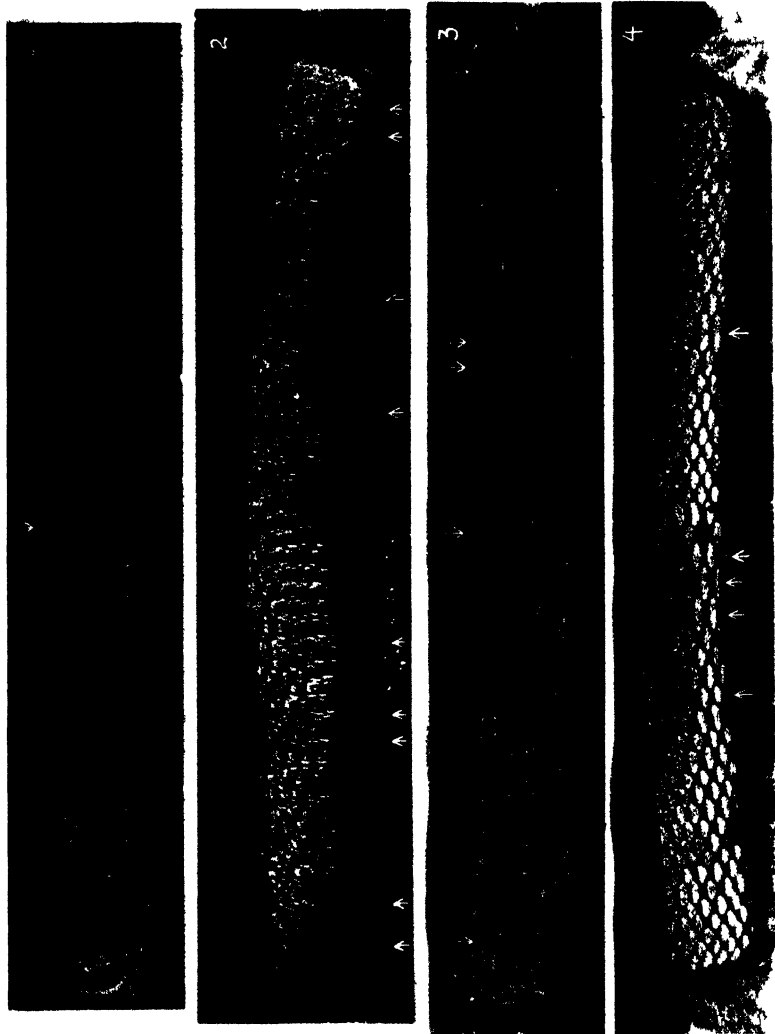
FIG. 1. Upper jaw of the male of the smaller devilfish, *Mobula hypostoma*, showing a marked abnormality in the symphysial (?) tooth row. U. S. National Museum no. 27653.

FIG. 2. Lower jaw of the same male fish (*Mobula hypostoma*) showing markedly abnormal tooth-rows marked by white arrows. U. S. National Museum no. 27653.

FIG. 3. Upper jaw of the female *Mobula hypostoma* showing abnormally broad rows of teeth marked with white arrows. U. S. National Museum no. 27654.

FIG. 4. Lower jaw of the same female *Mobula hypostoma* with abnormally wide tooth-rows marked with white arrows. U. S. National Museum no. 27654.

PLATE 2





## MODERN ZOÖGEOGRAPHY

By Z. P. METCALF

### FOUR TEXT FIGURES

In the decades immediately following the publication of the "Origin of Species" under the able leadership of Wallace and Sclater a great deal of work was done on the study of the distribution of animals. A definite science of Zoögeography was developed. Natural selection explained many hitherto inexplicable facts in distribution and on the other hand the study of zoögeography contributed so many facts to the support of the theory of natural selection that it is considered today as one of the cornerstones of evolution. Gradually students devoted themselves more and more to the study of the minutiae of distribution and thus was born the science of ecology. During the Twentieth Century students of distribution have devoted themselves more and more exclusively to the study of all phases of local distribution, the various factors in the environment, the types of adaptation to the environment and other phases of ecology to the almost complete exclusion of any consideration of the broader aspects of distribution. I want, however, to make a plea for the study of the broader aspects of distribution, zoögeography. We have much to learn from such a study. And the knowledge thus gained can contribute much to many other fields, notably evolution, taxonomy, and morphology. This is especially true in the study of the distribution of insects.

Insects constitute the vast majority of kinds of animals living in the world today. As near as I can estimate eight times as many species of insects have been described as of all other kinds of animals combined. But careful studies of a small order of rather inconspicuous insects would convince me that we do not know more than a tenth or perhaps a twelfth of the Homoptera of the world. If this ratio holds throughout and assuming that the larger and more conspicuous animals like the birds, fishes, mammals, and reptiles are proportionately better known, our ratio of living insects to all other kinds of animals combined is perhaps of the order of 50 to 1, 80 to 1, or perhaps an hundred fold. To comprehend this vast assemblage it will be necessary for us to broaden our base

not only in taxonomy and morphology but I believe in many other directions, and more particularly in the field of distribution. I make therefore a plea for the study of zoögeography. Not as an exact science. That to my mind was one of the great mistakes of the past. But zoögeography studied in a broad philosophical manner without too much attention to details has much to contribute to present day zoölogy.

If then you can forget for the moment that science is exact in all its measurements and consists only of test tubes, microscopes, micrometers, and all the other paraphernalia of the modern laboratory and come with

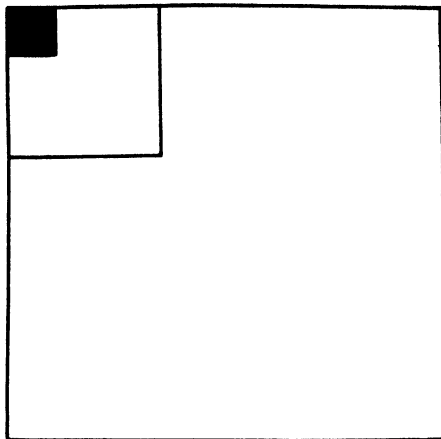


FIG. 1. DIAGRAM SHOWING RELATIVE NUMBER OF INSECTS IN COMPARISON WITH ALL OTHER ANIMALS

The large square shows the number of described insects; the small white square the number of all other animals combined; the small black square indicates all kinds of animals in comparison to the insects, assuming that only one-tenth of the living insects have been described.

me out on the mountain top where our vision is obscured by none of these, and where we can indulge in the fancies of the poet, the artist, and the philosopher, perhaps we can come back to our microscopes with clearer eyes and keener vision.

With this very rambling and somewhat fanciful introduction let us turn our attention to a consideration of the distribution of animals over the surface of the earth. There can be no doubt about the fundamental fact of zoögeography—namely, that animals are not uniformly distributed over the surface of the earth. Various schemes of zoögeographic classification have been proposed but none are entirely suc-

cessful. The scheme here proposed has its limitations but fits the facts of distribution of the insects of the Order Homoptera better than any other I have been able to devise. It is proposed in order that it may be tested critically by others for other groups and in this way we may arrive at a more satisfactory zoögeographical classification.

Zoögeography assumes that each species living in the world today has originated in a definite area known as the center of origin and has gradually spread until it occupies its present range. Species of recent origin would occupy restricted areas and would still be spreading. Thus the European starling which was introduced into this country about 1890 has spread along the Atlantic seaboard and has more recently crossed

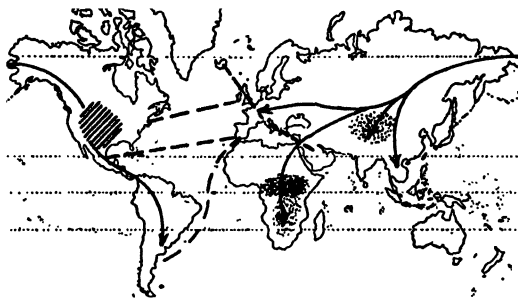


FIG. 2. MAP SHOWING THE ORIGIN AND MIGRATION OF HORSELIKE ANIMALS FROM A HYPOTHETICAL CENTER (SHADED AREA) IN WESTERN NORTH AMERICA

The present distribution of wild horses, asses and zebras is indicated by the dotted areas. The solid lines indicates probable routes of migration. The dashed lines indicate the distribution of horses to the Western Hemisphere by civilized man.

the Alleghany Mountains and seems destined to occupy the whole country. Species of older origin may have spread throughout the available range. The English sparrow introduced from Europe about 1850 has gradually spread until it is found throughout the entire country. Both of these illustrations are drawn from accidental introductions and these birds have spread at a much more rapid rate perhaps than is normal for most animals, but they illustrate the point forcibly. Groups of more ancient origin may have spread so far from their centers of origin that they have lost contact with their centers, and it may be possible to establish the centers of origin only through an examination of the fossil record. Thus, it is believed that the horses and camels originated in



western North America. From this center the horses spread to Asia and Africa, and the camel tribe is represented in Asia and Africa by the true camels and in the Andes of South America by the llamas and their relatives. Groups may be so isolated and so restricted that they are on the verge of extinction. The egg-laying mammals of the Australian regions may be taken as an example.

An animal spreading from a center of origin will find its spread facilitated in regions of uniform conditions. These regions may be called highways of dispersal such as the uniform coastal plains along the Atlantic Coast or the Great Plains region in the interior. In a lesser way streams and mountain passes may furnish highways across otherwise impassable barriers. Just as there are highways for the dispersal of

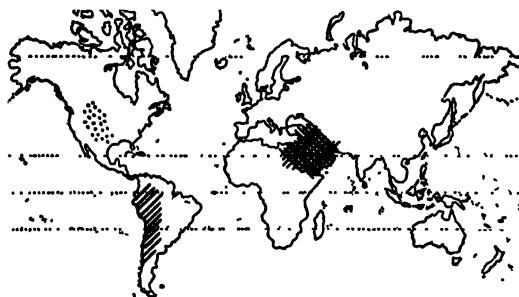


FIG 3. MAP SHOWING THE CENTER OF ORIGIN (DOTTED AREA) IN WESTERN NORTH AMERICA OF THE CAMEL-LIKE ANIMALS

The distribution of the recent llamas in South America and of the recent camels in Asia is indicated by the shading.

animals; so oceans, seas, lakes and rivers, mountain ranges and valleys, deserts and marshes may form barriers to their dispersal. The best means of dispersal at the command of most land animals are their own methods of locomotion.

In classifying the zoögeographic regions of the world we shall speak of superrealms, realms, subrealms, regions, and subregions just as in ordinary geography we speak of hemispheres, continents, countries, states, and counties. And just as the geographic boundaries are not hard and fast and are subject to changes and shifts, due to political fortunes, as witness the map of modern Europe as compared with the map of Europe in 1914; so these geographic boundaries which delimit the distribution of animals are subject to revision. I have attempted to indicate this in

the accompanying map by making the boundaries broad and in certain areas setting up neutral or buffer areas to indicate a condition of overlapping faunas or of zoögeographic question marks which will be settled only by more detailed study.

#### A PROPOSED CLASSIFICATION OF THE ZOÖGEOGRAPHIC REGIONS OF THE WORLD

<b>Superrealm Paragea</b>	<b>Neogaeen realm</b>
<b>Arctogaeen realm</b>	<b>Neotropical region</b>
<b>Holarctic subrealm</b>	Andean subregion
<b>Palearctic region</b>	Amazonian subregion
European subregion	Pampean subregion
Mediterranean subregion	Orinocian subregion
Siberian subregion	<b>Caribbean region</b>
Mongolian subregion	Aztecian subregion
Japanese subregion	Mayan subregion
<b>Neartctic region</b>	Antillian subregion
Canadian subregion	<b>Superrealm Telegea</b>
Alleghanian subregion	<b>Notogaeen realm</b>
Cordilleran subregion	<b>Australian region</b>
Californian subregion	Carpentarian subregion
<b>Paleotropical subrealm</b>	Victorian subregion
<b>Ethiopian region</b>	Aurrian subregion
Mozambican subregion	Tasmanian subregion
Caffrarian subregion	<b>Austromalayan region</b>
Guinean subregion	Papuan subregion
Sudanese subregion	Melanesian subregion
Malagasian subregion	<b>Nesogaeen realm</b>
<b>Oriental region</b>	<b>Maorian region</b>
Indian subregion	<b>Oceanic region</b>
Ceylonese subregion	Polynesian subregion
Siamese subregion	Micronesian subregion
Malaysian subregion	Hawaiian subregion
Philippines subregion	
Celebesian subregion	

#### **Superrealm Paragea**

This superrealm comprises about 95 per cent of the land area of the world. It includes the continents of Europe, Asia, Africa, North and South America, and the adjoining islands. It is characterized principally by the dominance of placental mammals, flying birds, fresh-water turtles, and terrapins, the dominance of the Colubrinae and Viperidae among the snakes. In the Amphibia the true frogs and true toads are present over much of this superrealm.

## ARCTOGAEAN REALM

This realm includes about 80 per cent of the land area of the globe, excluding the Antarctic Continent which is practically uninhabited by land animals. This realm includes all of Europe and the adjacent islands; all of Africa and the adjacent islands including Madagascar; all of Asia and the adjacent islands, Japan, the Philippines, the East Indies as far as Celebes and Ceylon; and North America as far south as Mexico. In this vast stretch of land, it is very difficult to find animals that are characteristic of the whole realm. The family Bovidae with its numerous genera is confined to this realm, and the different species are distributed over practically all this realm or were before man eliminated certain of the larger species from certain areas. This realm includes all the hoofed mammals, all the Proboscidea, and practically all the Insectivora. It is destitute of Edentata and has no Marsupialia

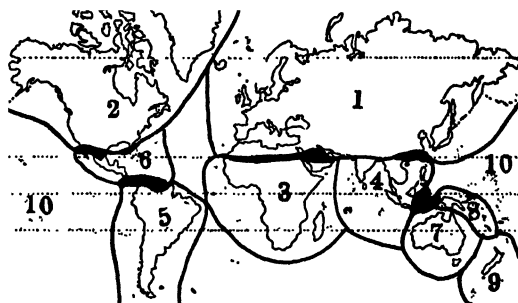


FIG. 4. MAP SHOWING THE ZOOGEOGRAPHIC REGIONS OF THE WORLD

1, Palearctic; 2, Nearctic; 3, Ethiopian; 4, Oriental; 5, Neotropical; 6, Caribbean; 7, Australian; 8, Austromalayan; 9, Maorian; 10, Oceanic.

save the North American opossum. Among the birds the only forms characteristic of the region as a whole are the loons or divers.

*Holarctic Subrealm*

This area includes North America north of Mexico, all of Europe, Asia north of the Himalayan Mountains, and Africa north of the Sahara Desert. In this area there are many animals that are circumpolar in their distribution, facing out to the southward. This includes such forms as the musk ox, the moose, the reindeer, the wolverine, the walrus, weasels, ermines, the polar hares or varying hares, and the lemmings

among the mammals and the ptarmigans among the birds. The beavers were formerly distributed in the Holarctic subrealm as are the loons among the birds. This subrealm is characterized by the presence of lynxes, foxes, deer, sheep, squirrels, the true bears, and marmots among the mammals. Some groups are represented by forms in Eurasia and related forms in North America—the red deer and the American elk, the two species of bison, for example. The tailed amphibia are found practically throughout this subrealm.

*Paleartic Region.* This region includes all of Europe and the adjacent islands, most of Asia save India and the Malay Peninsula, and Africa north of the Sahara Desert. The Himalayan Mountains and the Arabian and Sahara Deserts seem to form an effective barrier between this region and the Palearctic subrealm to the south. Physiographically this is a rather uniform area. The mountain ranges are generally short and are not effective barriers. The western area is rather densely wooded. A considerable extent along the southern border of this region is desert; this embraces parts of the Sahara and Arabian Deserts and all of the Mongolian Desert. The northern part of the region consists principally of lowland steppes. This region is the center for the development of the sheep and goats. Many different species occupy isolated areas such as mountain ranges and islands throughout this region. This is the region of the pikas of the higher mountains and the Old World rats and mice, the genus *Mus* of about 130 species. Among the reptiles the common viper is widely distributed in this region and is a dangerously poisonous snake.

*Nearctic Region.* This region includes Greenland and North America south to Mexico. It is a region with extensive forests and open treeless plains. The desert regions are small. The mountain ranges extend north and south chiefly and are not important barriers. Its chief characteristic mammals which cover considerable areas are the raccoon, the opossum, the jumping mice, the pocket gophers, the skunks, and the muskrat. The ungulates are poorly represented as there are no horses nor pigs, save domestic forms introduced by man. The buffalo or bison and the elk were formerly widely distributed. The bighorn sheep and the pronghorn antelope are characteristic but they occupy only restricted areas. Among the birds, turkeys, blue jays, and buzzards are quite characteristic but range southward into the Neotropical Region. Among the reptiles the rattlesnakes are the most characteristic forms.

*Paleotropical Subrealm*

This subrealm includes Africa south of the Sahara, Madagascar, India, and the Malay Peninsula and the larger East Indian Islands. It is varied in its topography and physiography but contains large areas of dense forests with heavy rainfall. This subrealm, though separated into an eastern and western region by the Indian Ocean, contains many groups of animals not found to any extent in other regions. The great cats, like the lion and leopard, the anthropoid apes, the elephants, and the rhinoceroses are confined to this subrealm. Among the reptiles the cobras, including the world's most deadly poisonous snakes, are confined to this subrealm.

*Ethiopian Region.* This region includes Africa and Arabia south of the great deserts, and the island of Madagascar which is sometimes ranked as a distinct region. The Ethiopian region is a region of great contrasts ranging from the largest deserts in the world to huge rain forests; from tropical climate to cold temperate; and from great snow-capped mountains to vast open veldts. It includes within its range many strange animals. The gorilla, the chimpanzee, the baboons, and the lemurs are representative primates. This region is the center for the development of the antelopes and the zebras as the majority of the species of these two groups are found in this region. Other curious mammals which are native to this region include the giraffe, the hippopotamus, and the hyrax. In contrast to these strange forms there is an almost total absence of bears, deer, oxen, wolves, and true foxes. The birds of this region include the peculiar guinea fowls and the secretary birds.

*Oriental Region.* This region consists of India, the Malay Peninsula, and the larger East Indian Islands, Borneo, Sumatra, Java, and the Philippines. It is a region characterized by dense jungles and heavy rainfall. Its characteristic animals include the orang-utan, the gibbons, the Indian tapir, the true buffalo among the mammals, and the peacock and the jungle fowl among the birds.

## NEOGAEAN REALM

This realm includes two regions, the Caribbean and the Neotropical. It is one of the marvels of zoögeography that this region which is not separated from the Nearctic region by any important barriers should differ so markedly while the Nearctic region which is separated by evident barriers from the rest of the Arctogaeon realm yet has so many things in common with that realm. The answer is found apparently in

the fact that this realm has been separated from the Nearctic region until fairly recent times, geologically speaking, whereas the Arctogaeian realm has been united into one great land mass until fairly recent times.

*Caribbean Region.* For the present at least I am separating this region from the rest of the Neogaeian Realm. This separation may not be sustained when all the facts are known. As at present constituted it consists of the lowlands of Mexico, Lower California, Central America, and the West Indies. The boundary lines to the north are fairly well established by the Straits of Florida, rather poorly defined by the highlands of Mexico. To the south the boundaries are not clear; perhaps the Isthmus of Panama; perhaps they must be moved further south to include the great valley of the Orinoco River. If this is a region and not a subregion it is characterized chiefly by certain species of mammals which have near relatives in South America and by certain insects.

*Neotropical Region.* This is a region of extensive lofty mountains, vast tropical forests, and unforested table lands. There are also great grassy plains in the north, the llanos, and in the south, the pampas. About two-thirds of the area is in the tropics. This region is characterized by a number of peculiar small mammals including the prehensile-tailed monkeys, the marmosets, ant-eaters, sloths, armadillos, peccaries, caviés, agoutis, llamas, alpacas, many kinds of opossums, and the American tapir. It is also characterized by the absence of oxen, sheep, horses, and deer, or other large mammals. The peculiar birds include the rheas, the motmots, and the vast majority of the humming birds.

### Superrealm Telegea

This superrealm includes the continent of Australia and the adjacent islands such as New Guinea and New Zealand and the many small islands of the Pacific. It is characterized by the dearth of placental mammals and the dominance of the marsupials and egg-laying mammals; by the dominance of the flightless birds and by the dominance of the Elaphinae snakes among the reptiles. For convenience we shall divide it into two realms—Notogaeian, including the Austromalayan and Australian regions, and Nesogaeian, including the Maorian and Oceanic regions.

#### NOTOGAEIAN REALM

This realm includes the islands of Celebes and New Guinea and the continent of Australia. The egg-laying mammals, monotremes, are

characteristic and the marsupials have developed to a greater extent than in any other region of the world. Among the birds are the flightless cassowaries and emus and the mound builders, brush turkeys, and lyre birds.

#### NESOGAEAN REALM

This realm includes many isolated islands, the largest being the New Zealand Islands. The rest are small, widely scattered over the Pacific Ocean and chiefly of volcanic origin. This realm is characterized by the almost complete absence of terrestrial mammals, save introduced forms, and of snakes, turtles, crocodiles, and amphibia. Among the birds the flightless kiwis are confined to New Zealand and the other islands are principally inhabited by sea birds.

#### CONCLUSIONS

In conclusion let me call your attention to the relation of these zoögeographic regions to taxonomy. Considering the facts from the broad general aspects only and disregarding the details for the moment we can arrange a parallel classification about as follows.

##### *Zoögeographic regions*

Superrealm

Realm

Region

Subregion

##### *Taxonomic groups*

Families

Subfamilies

Genera and Subgenera

Species and Subspecies

This classification if correct means that it should work both ways. Superrealms should be defined by families and genera should be confined to regions, species to subregions *et cetera*. Now such a broad generalization does not mean that some species do not have a broader distribution than a subregion, or that some genera are not distributed over two, three, or more regions. Some species are found in more than one region, in some cases being distributed by man in connection with his cultivated crops. Thus the corn plant hopper (*Peregrinus maidis* Ashmead) was described from Florida, and occurs in the southern states from North Carolina to Texas, and has been found wherever maize is grown in the tropical and warm temperate regions of the world, Ceylon, Hawaii, Queensland, Fiji, Java, throughout the West Indies, India, New South Wales, Mexico, Nicaragua, Brazil, Nigeria, Philippine Islands, Formosa, Malay Peninsula, Amboyna, Borneo, Natal, and Polynesia. Certain genera have a wider distribution than a single zoögeographic region. What I am trying to say is that the taxonomist and systematist should

look with suspicion on all such cases of wide distribution especially of uncorrelated distribution. Genera may have isolated species in South America and in India or in China and South Africa, but such cases are open to very grave suspicion. There may be cases of genera having different species which have lost the connections with their center of origin as in the case of the camel tribe discussed above but such cases should only be established by careful consideration of all the facts. At the present time we have many genera especially the older established genera which were very vaguely defined and which have become the dumping ground for a vast host of species simply because the generic descriptions are too all inclusive. For example the genus *Delphacodes* Fieber as at present constituted contains no less than 48 species in the Nearctic Region, 105 species in the Palearctic Region, 17 species in the Ethiopian Region, 13 species in the Oriental Region, 19 species in the Caribbean Region, 40 species in the Neotropical Region, 6 in the Australian Region, 1 species in the Austromalayan Region, 1 species in the Maorian Region, and 5 species in the Oceanic Region. Now, I have no more reason for believing that the genus *Delphacodes* represents the correct systematic position of these 255 species than that the genus *Cicada* represented the correct position of the 42 species described by Linné in 1758. These 42 species are today distributed among 9 families and 16 genera. The correct solution of such taxonomic problems, as presented by the complex genus *Delphacodes*, awaits a better understanding of taxonomic characters, zoögeographic distribution, *et cetera*. It is of course barely possible that this genus is a very ancient one of world wide distribution but it is certainly a safer assumption that it represents a vague complex with poorly defined and very generalized characters of polyphyletic origin. All such cases should be carefully reconsidered and the boundaries of such taxonomic groups should be redefined and modernized. By these and similar methods only will it be possible to make taxonomy a more exact science.

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## OCCAM'S RAZOR AND MENDEL'S PEAS

By J. P. GIVLER

"The Invincible Doctor," William of Occam, the famed opponent of the temporal power of the popes, is commonly credited by students of the history of philosophy and of science with the discovery of a simple principle upon which all modern science rests. This dictum, known as the "Law of Parsimony," states that "entities are not to be multiplied beyond necessity." It has become known as "Occam's Razor." According to the nominalistic philosophy, which Occam revived in Europe, "only individual things exist. Universals are not realities, but only inferences of the thinking mind." This late mediaeval scholastic doctrine of the Franciscan pupil of Duns Scotus has become fundamental for the logic of science.

In striking contrast to the logical basis given by Occam to modern science there has flowed from the empirical studies of another Catholic churchman, Gregor Mendel, an Augustinian monk, two simple principles upon which our entire modern science of genetics has been founded. From the development of these have come practical applications of great commercial value, a recent explanation of organic evolution, and indeed, a pretentious theory of the organism. The history of modern genetics covers only three decades, but it is involved with both technical and philosophical considerations. It is concerned with crucially planned programs of breeding experiments, on the one hand, and penetrating studies of the nucleus, especially of the germ-cells, on the other. Its theories rest on the relationships have been found, or which have been inferred, to exist between these two fields of precise study. In all of this work which has sprung from Mendel's studies, the "Rule of Parsimony" has been applied to the letter. The metaphysician of the thirteenth century dictated canons of thought to the Abbot of Brunn, the empirical experimenter, of the nineteenth, as well as to the army of Neo-Mendelians of the twentieth. A reading of Mendel's original paper on the hybridization of peas shows with what aplomb he chose the materials for his experiments and, further, how he chose with judicial detach-

<sup>1</sup> *The New Universal Encyclopedia.*

ment the very characters upon which his experiments were to focus attention. Upon these his interest remained fixed through seven years of meticulous toil, and it is well known to every student of genetics that this is the reason why he succeeded where John Goss, Naudin, and a host of former hybridizers failed. In a word, Mendel succeeded because he applied Occam's Razor and with it ruthlessly cut off all "entities" extraneous to his pre-delineated problem. In the collection and statement of his results we likewise see the selective action of his mind. According to Singer<sup>2</sup> the productive scientist simply *must* be a "learned chooser," a fact which Bacon, the founder of the Inductive Method, failed to realize.

Since the rediscovery of Mendel's principles in 1900, and as the result of the parallel development of breeding experiments and upon chromosomes (karyology), the entire science of biology has been imbued with a new spirit. The various biological provinces such as heredity, taxonomy, embryology, and even physiology, palaeontology, and pathology have come under the domination of a set of principles having their origin about seventy years ago in the work of Mendel, and known as "The Theory of the Gene." It is known to every working biologist and to a great many laymen that the advance of this empire of the gene into the various biological kingdoms compares with the advance of the empires of Alexander or of Caesar and is therefore the most signal development in the science of life since the publication of Darwin's *The Origin of Species* in 1859. Under these circumstances all detached thinkers should examine the Mendelian edifice to ascertain the nature both of its material and of its philosophical basis, the justification for its very inclusive claims, its relation to life as a planetary phenomenon rather than as a subject for laboratory experimentation, and the probabilities of its permanency. Only the professional geneticist can fully realize the scope of such an undertaking. The intricacy of empirical method, the highly specialized technique, nomenclature, and reasoning processes render the field a practically closed one to all except the acolytes of the genetic temple, and there are occasional intimations that its high priests have been a party to this esoteric situation.

As a chapter in the current history of biology the development of genetics in the present day is one which is impossible of comprehension on sufficiently broad lines to be of general service without reference to its logical and philosophical background. That the movement has any

<sup>2</sup> Singer, Charles. *The Story of Living Things*, p. 121. New York.

such foundation most of the empiricists concerned with it would emphatically deny. They commonly refer to their opponents as both "philosophers" and "metaphysicians" without realizing the history and nature of their own logical instruments. Genetics has a firm basis in logic, although its foundations are narrow and not invulnerable. The detached student sometimes gains the impression that wild and domesticated faunas and floras have been "ransacked" to find incisively diagrammatic and cogent evidence. The theory of the gene is an outline of a definite biological philosophy and also a recrudescence of the atomistic<sup>3</sup> philosophy of Leucippus and Democritus of 400 B.C. which had further elaboration by the Epicureans, especially by Lucretius. With the latter it became a perfect system of materialism. "Democritus held that even the Soul consists of fine, round and smooth atoms!" The Augustinian Monk of the nineteenth century, heeding the instructions of his brother churchman of the thirteenth, selected both his organism and the contrasting characters on which he was to keep watch through several years with the most stringent "parsimony," for he rigidly excluded all other factors and, in so doing, succeeded where his predecessors failed. He used Occam's razor to shear off "unessentials." In so doing it becomes an open question whether Mendel was a biologist and his experiments biological ones. In the usual meaning of the term I am personally somewhat against both conclusions.

Two of Mendel's biographers<sup>4</sup> reveal that the Abbot's scientific education at the University of Vienna was short, as it extended only through the two years between 1851 and 1853, and that it leaned heavily toward mathematics and physics. In natural history Mendel was very largely self-taught, which in part accounts for the fact that he failed in an examination of university grade in that subject under Professor Kner. At Brünn he became a successful teacher, especially of physics, in the Realschule. But nowhere is there any evidence that he was a man

<sup>3</sup> It is worthy of note that, in the attempt to place biology on a thoroughly "scientific" basis, it is repeating the history of chemistry, which employed the concept of the atom until the beginning of the present century. Practically speaking, the gene is the biological atom, i.e., the uncuttable, as is witnessed by such conceptions as that of "the purity of the gametes," prevalent since the first statement of Mendel's theoretical views. Recent discussions of "protogenes" and of the significance of the "step mutations" of the Russian geneticists Serebrovsky and Dubinin, however, would seem to indicate that the gene has already begun to follow the course of the atom.

<sup>4</sup> Bateson, William, *Mendel's Principles of Heredity*, and Iltis, Hugo, *Life of Mendel*.

broadly trained in botany and much less that he had the naturalist's view of the plant as a whole in its relations to environment, such as we find in Kerner. In the judgment of the present writer Mendel was an independent, plodding genius, unfortunately in advance of his time, in whom a brief academic training and traditions in the logic of ecclesiastical dialectic had prepared him for his initial and penetrating analysis of hybridology. With his training in physics and pure mathematics he was able to conceive and envision his problem as though the factors involved were points without extension, played upon by the hand of probability. The abundant results, both theoretical and practical, which have flowed from his work are known to all. They range from hornless cattle and the Shasta daisy to the measurement of the distances between genic loci in chromosomes. With little knowledge of microscopy he proposed laws which transcend the reach of the lens into living matter, but again, whether his was a biological approach or not, remains a question of definition. The biometrician, applying involved formulae in the study of his data, is perhaps a biologist but we are warned by Johannsen that we must pursue our quest "with the *aid* of mathematics and not *as* mathematics." The point I raise here is one that appears to me both crucial and also basic to the study and evaluation of the entire Mendelian superstructure—is the study of an excised aspect of the complex phenomena of a living species to be regarded in the same light as studies of the whole organism in relation to all of its environment? Such broadminded thinkers as Brooks did not think so. Whitman was cautious in his acceptance of such philosophy and H. F. Osborn is not only suspicious but more than mildly inimical.

The geneticists' appeal to the pure claims of discrete evidence are very strong: they would give us to believe that they "have no brains above their eyes." But this claim is clearly false, for their work is obviously permeated with the belief that living nature is essentially simple and that "her much-advertised inscrutability has once more been found to be an illusion due to our ignorance."<sup>5</sup> This is not only philosophy, from which the majority of accepted geneticists claim to have been purged, but it is also an unwarranted assumption uttered in the face of portentous evidence to the contrary.

While it is impossible here to point out many aspects of modern genetical theory in its relation to this discussion, I wish to call your attention to the inadequacy of the supposedly simple mechanistic explanations

<sup>5</sup> Morgan, T. H., 1919, *The Physical Basis of Heredity*, p. 15.

which have been offered in a few cases. From McClung's suggestion in 1902 that the accessory chromosome is the "sex determinant" came at first the general belief that this complex factor in life hinged on the presence or absence of a single chromosome. As time went on, the work in polyploidy and in the study of intersexes showed that the sex of the individual "is turned this way or that as the result of a quantitative balance between X-chromosomes and autosomes" (E. B. Wilson). It was, by the way, the work on linkage in the sex chromosome that led to our theory of gene distances and to the making of gene maps. This at first seemed to point to very simple parallels between the supposedly mosaic character of the organism and the strings of genes in or on the chromosomes. But the facts of both breeding and cytology arise to mock the investigator. As with the case of red eye color in *Drosophila*, the fruit-fly, reviewed by Jennings,<sup>6</sup> many particular "unit characters" may have genes, as he states it, "all over the lot," and the simple relations of genes to somatic characters envisioned by college undergraduates proves a delusion born of academic first impressions. There is some "law within the law" beyond all this that nobody as yet perceives. The situation calls for the emergence of a man with the Faraday type of mind—the intellect that ranged ahead of his problem and converted the tenuous tissue of scientific imagination into concrete laws of reality. Such a new super-Mendelism may resolve our present confusion.

It appears from the above examples of modern genetical work at the very firing-line that the result of carrying Mendel's rules to the furthest limit is not, within our generation, to resolve the factors of life and evolution to simplicity. That genetical work has been abundantly justified from both theoretical and practical standpoints there can be no doubt, but that it leads us to a simple explanation of life is a conclusion apparently unjustified by fuller knowledge.

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<sup>6</sup> Jennings, H. S., *The Biological Basis of Human Nature*, p. 193.

## NOTES FROM TEN YEARS OF BIRD STUDY AT GREENSBORO, NORTH CAROLINA

By EARL H. HALL

It is the purpose of this paper to present to the beginning student in particular, and to the advanced student of ornithology as well, as complete a catalog of the birds of Greensboro community as possible. Since the locality is typical of the piedmont, the list covers a wide range. This work has been compiled from personal records covering the past ten years and checked with the observations of several amateurs who have zealously followed the sport of making bird walks. I am indebted to Mr. Joe H. Armfield, Mr. Lacy McAlister, and Mr. Robt. D. Douglas for their aid, enthusiasm, and companionship in making this study. I also used State Bulletin No. 144, "Ornithology of North Carolina," as a check list.

Observations made in handling beginners have led me to believe that they would profit by having a complete list of our birds arranged in some simple order. Such a list would enable a beginner to know his limitations, as well as to measure his progress and ability against those students who have advanced in the study of birds. Besides, this condensed information would aid in assuring—without guessing—as to what birds are in the community.

I shall make no attempt to show relationships or even to hint at any scientific classification; nor shall I deal with the habits or activities of the birds. The interest in bird study must come entirely from the student who, equipped with this guide, may assist the author in making a complete catalog of the local birds. Then, as the student becomes more interested, he may add, by his own research, to his knowledge concerning the habits, customs, or any other activities that suit his fancy. However, for those who wish to make further study of the subject I have placed in parentheses the scientific name of each species.

This list should appeal particularly to the person who cares little for technical ornithology, but who enjoys having the birds about his home and learning more about them. The subject of birds is of never failing interest to the bird lover, and his interest increases as he reads or studies their movements, or has an opportunity to compete with a fellow stu-

dent. In attempting to simplify matters, I have arranged the hundred and forty-one birds into three groups: group I, consisting of birds that are common and would be seen by the casual observer; group II, of birds not so frequently seen; and group III, of birds that are rare or difficult to observe. The lists follow:

#### GROUP I: FOR THE CASUAL OBSERVER

- |  |  |
|--|--|
| 1. American Goldfinch, Lettuce Bird<br>( <i>Astragalinus tristis</i> ) | 14. Flicker, Yellow-hammer<br>( <i>Colaptes auratus</i> )                |
| 2. Black-capped Chickadee<br>( <i>Parus atricapillus</i> )             | 15. Meadowlark, Fieldlark<br>( <i>Sturnella magna</i> )                  |
| 3. Blue Bird<br>( <i>Sialia sialis</i> )                               | 16. Mockingbird<br>( <i>Mimus polyglottos</i> )                          |
| 4. Blue Jay<br>( <i>Cyanocitta cristata</i> )                          | 17. Mourning Dove<br>( <i>Macroura carolinensis</i> )                    |
| 5. Bobwhite, Partridge, Quail<br>( <i>Colinus virginianus</i> )        | 18. Nighthawk, Bullbat<br>( <i>Chordeiles virginianus</i> )              |
| 6. Brown Thrasher<br>( <i>Toxostoma rufum</i> )                        | 19. Red-headed Woodpecker<br>( <i>Melanerpes erythrocephalus</i> )       |
| 7. Cardinal<br>( <i>Cardinalis cardinalis</i> )                        | 20. Robin<br>( <i>Planesticus migratorius</i> )                          |
| 8. Carolina Chickadee<br>( <i>Parus carolinensis</i> )                 | 21. Ruby-throated Hummingbird<br>( <i>Archilochus colubris</i> )         |
| 9. Catbird<br>( <i>Dumetella carolinensis</i> )                        | 22. Slate-colored Junco, Snowbird<br>( <i>Junco hyemalis</i> )           |
| 10. Chimney Swift<br>( <i>Chaetura pelagica</i> )                      | 23. Starling<br>( <i>Sturnus vulgaris</i> )                              |
| 11. Chipping Sparrow<br>( <i>Spizella passerina</i> )                  | 24. Turkey Vulture, Buzzard<br>( <i>Cathartes aura septentrionalis</i> ) |
| 12. Crow<br>( <i>Corvus brachyrhynchos</i> )                           | 25. Wood Thrush<br>( <i>Hylocichla mustelina</i> )                       |
| 13. English Sparrow<br>( <i>Passer domesticus</i> )                    | 26. Whip-poor-will<br>( <i>Antrostomus vociferus</i> )                   |

#### GROUP II: FOR THE AMATEUR OBSERVER

- |   |  |
|---|--|
| 1. American Redstart<br>( <i>Setophaga ruticilla</i> )      | 7. Blue-headed Vireo<br>( <i>Lanivireo solitarius</i> )          |
| 2. Belted Kingfisher<br>( <i>Ceryle alcyon</i> )            | 8. Bronze Grackle<br>( <i>Quiscalus quiscula aeneus</i> )        |
| 3. Black-poll'd Warbler<br>( <i>Dendroica striata</i> )     | 9. Brown Creeper<br>( <i>Certhia familiaris americana</i> )      |
| 4. Black Vulture<br>( <i>Catharista urubu</i> )             | 10. Carolina Wren<br>( <i>Thryothorus ludovicianus</i> )         |
| 5. Black and White Warbler<br>( <i>Mniotilta varia</i> )    | 11. Cedar Waxwing<br>( <i>Bombycilla cedrorum</i> )              |
| 6. Blue-gray Gnatcatcher<br>( <i>Poliophtila caerulea</i> ) | 12. Chestnut-sided Warbler<br>( <i>Dendroica pennsylvanica</i> ) |

13. Crested Flycatcher  
(*Myiarchus crinitus*)
14. Downy Woodpecker  
(*Dryobates pubescens*)
15. Field Sparrow  
(*Spizella pusilla*)
16. Fox Sparrow  
(*Passerella iliaca*)
17. Golden Crowned Kinglet  
(*Regulus satrapa*)
18. Great Blue Heron  
(*Ardea herodias*)
19. Green Heron  
(*Butorides virescens*)
20. Hairy Woodpecker  
(*Dryobates villosus*)
21. House Wren  
(*Troglodytes aedon*)
22. Indigo Bunting  
(*Passerina cyanea*)
23. Killdeer  
(*Aegialitis vocifera*)
24. Kingbird  
(*Tyrannus tyrannus*)
25. Little Blue Heron  
(*Ardea caerulea*)
26. Maryland Yellow-throat  
(*Geothlypis trichas*)
27. Myrtle Warbler  
(*Dendroica coronata*)
28. Palm Warbler  
(*Dendroica palmarum*)
29. Pied-billed Grebe  
(*Podilymbus podiceps*)
30. Phoebe  
(*Sayornis phoebe*)
31. Pine Warbler  
(*Dendroica vigosii*)
32. Prairie Warbler  
(*Dendroica discolor*)
33. Purple Finch  
(*Carpodacus purpureus*)
34. Purple Grackle  
(*Quiscalus quiscula*)
35. Purple Martin  
(*Progne subis*)
36. Red-eyed Vireo  
(*Vireosylva olivacea*)
37. Red-winged Blackbird  
(*Agelaius phoeniceus*)
38. Ruby-crowned Kinglet  
(*Regulus calendula*)
39. Scarlet Tanager  
(*Piranga erythromelas*)
40. Song Sparrow  
(*Melospiza melodia*)
41. Spotted Sandpiper  
(*Actitis macularia*)
42. Summer Tanager  
(*Piranga rubra*)
43. Towhee  
(*Pipilo erythrophthalmus*)
44. Tufted Titmouse  
(*Parolophus bicolor*)
45. Vesper Sparrow  
(*Pooecetes gramineus*)
46. White-breasted Nuthatch  
(*Sitta carolinensis*)
47. White-throated Sparrow  
(*Zonotrichia albicollis*)
48. Wood Pewee  
(*Myiochanes virens*)
49. Yellow-bellied Sapsucker  
(*Sphyrapicus varius*)
50. Yellow-throated Vireo  
(*Lanivireo flavifrons*)
51. Yellow Warbler  
(*Dendroica aestiva*)

## GROUP III: FOR THE HIGHLY TRAINED OBSERVER

1. Bachman's Sparrow  
(*Peucaea aestivalis bachmani*)
2. Baltimore Oriole  
(*Icterus galbula*)
3. Barn owl  
(*Strix pratineola*)
4. Barn Swallow  
(*Hirundo erythrogastra*)
5. Barred Owl  
(*Syrnium nebulosum*)
6. Bay-breasted Warbler  
(*Dendroica castanea*)



7. Black-crowned Night Heron  
(*Nycticorax nycticorax naevius*)
8. Black-throated Blue Warbler  
(*Dendroica caerulescens*)
9. Black-throated Green Warbler  
(*Dendroica virens*)
10. Blackburnian Warbler  
(*Dendroica fusca*)
11. Blue Grosbeak  
(*Guiraca caerulea*)
12. Blue-winged Warbler  
(*Vermivora pinus*)
13. Bobolink  
(*Dolichonyx oryzivorus*)
14. Brown-headed Nuthatch  
(*Sitta pusilla*)
15. Canadian Warbler  
(*Wilsonia canadensis*)
16. Cape May Warbler  
(*Dendroica tigrina*)
17. Cerulean Warbler  
(*Dendroica cerulea*)
18. Chuckwill's Widow  
(*Antrostomus carolinensis*)
19. Cooper's Hawk  
(*Accipiter cooperi*)
20. Cowbird  
(*Molothrus ater*)
21. Florida Gallinule  
(*Gallinula galeata*)
22. Grasshopper Sparrow  
(*Ammodramus savannarum australis*)
23. Great Horned Owl  
(*Bubo virginianus*)
24. Henslow Sparrow  
(*Passerherbulus henslowi*)
25. Hermit Thrush  
(*Hylocichla guttata pallasii*)
26. Hooded Warbler  
(*Wilsonia citrina*)
27. Horned Lark  
(*Otocoris alpestris*)
28. Lesser Scaup  
(*Aythya affinis*)
29. Loggerhead Shrike  
(*Lanius ludovicianus*)
30. Long-billed Marsh Wren  
(*Telmatodytes palustris*)
31. Louisiana Water Thrush  
(*Seiurus motacilla*)
32. Magnolia Warbler  
(*Dendroica magnolia*)
33. Mallard Duck  
(*Anas boschas*)
34. Olive-backed Thrush  
(*Hylocichla ustulata swainsoni*)
35. Oven-bird  
(*Seiurus aurocapillus*)
36. Parula Warbler  
(*Compsothlypis americana*)
37. Philadelphia Vireo  
(*Vireosylva philadelphia*)
38. Pileated Woodpecker  
(*Phloeotomus pileatus*)
39. Pine Finch  
(*Spinus pinus*)
40. Prothonotary Warbler  
(*Protonotaria citrea*)
41. Red-bellied Woodpecker  
(*Centurus carolinus*)
42. Red-breasted Nuthatch  
(*Sitta canadensis*)
43. Red-shouldered Hawk  
(*Buteo lineatus*)
44. Red-tailed Hawk  
(*Buteo borealis*)
45. Rusty Blackbird  
(*Euphagus carolinus*)
46. Sharp-shinned Hawk  
(*Accipiter velox*)
47. Savannah Sparrow  
(*Passerculus sandwichensis savanna*)
48. Screech Owl  
(*Megascops asio*)
49. Sparrow Hawk  
(*Falco sparverius*)
50. Swamp Sparrow  
(*Melospiza georgiana*)
51. Tree Sparrow  
(*Spizella monticola*)
52. Virginia Rail  
(*Rallus virginianus*)
53. Water Thrush  
(*Seiurus noveboracensis*)
54. Warbling Vireo  
(*Vireosylva gilva*)

- |  |   |
|--|---|
| 55. White-eyed Vireo<br>( <i>Vireo griseus</i> )           | 60. Wood Cock<br>( <i>Philohela minor</i> )                             |
| 56. Wild Turkey<br>( <i>Meleagris gallopavo</i> )          | 61. Worm-eating Warbler<br>( <i>Helminthos vermivorus</i> )             |
| 57. Wilson Snipe<br>( <i>Gallinago delicata</i> )          | 62. Yellow-billed Cuckoo<br>( <i>Coccyzus americanus</i> )              |
| 58. Wilson's Thrush, Veery<br>( <i>Turdus fuscescens</i> ) | 63. Yellow-breasted Chat<br>( <i>Icteria virens</i> )                   |
| 59. Winter Wren<br>( <i>Nannus hiemalis</i> )              | 64. Yellow-headed Blackbird<br>( <i>Xanthocephalus xanthocephalus</i> ) |

It is desirable to keep some form of bird record if for no other reason than to check your own observations. Our memories are rather fickle unless we have concrete evidence to check our thinking. These records of the spring arrival of birds differ with the individuals whose interest varies in bird study. Some merely keep a list of the first arrivals; others maintain that a daily list is better; still others make a special effort to record the first females to come and the beginning of the nest for each species nesting in that community.

Each year the members of my household vie with each other in keeping a calendar of the arrival of the birds on our grounds. It was begun in a spirit of play, but now I find that we have amassed much interesting information about our birds. By comparing the past dates of arrivals we are able to predict to almost the day when each species will be on the grounds. We also know that the Chipping Sparrow does not begin his nest until at least three weeks after the first bird has been seen. Even the resident Mockingbird does not begin nesting until about the last week in April. Time and space would not permit the enumeration of the list as it is kept from season to season. I will submit only a short list to show the regularity of some of our early visitors:

	ARRIVAL	EARLY	LATE	RECORD
Brown Thrasher.....	March 30	2	1	1925-1933
Wood Thrush.....	April 15	1	1	1923-1933
Red-eyed Vireo.....	April 20	1	0	1926-1933
House Wren.....	May 2	1	2	1925-1933
Catbird.....	May 4	2	0	1923-1933

This will suffice to illustrate one method of recording, but sooner or later as one learns the arrival time and knows the place where each species nests one really becomes interested in the arrival of individual birds. To be sure of this would necessitate bird banding, a method that

not every individual can practice. However, let every one gather such facts of our common birds as will enable us to determine whether males precede the females or whether old birds precede those hatched the previous year, and whether migrants pass through before residents arrive. Let us secure data on each species before we conclude that all species do as some one species has behaved in the past.

The author invites criticism and suggestions, especially from those who have had experience in conducting classes in bird study, or those who enjoy watching birds.

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## PARASITES AND COMMENSALS OF NORTH CAROLINA CRAYFISHES

By SALLY ANDERSON ALLEN

### PLATE 3

In order to study the animals associated with crayfishes in North Carolina, specimens of *Cambarus acuminatus* Faxon were collected from Hibbard's pond and the old city reservoir on the Eno River near Durham in September, and specimens of *Cambarus blandingii* Harlan from the streams near Charlotte in November. Dr. Edwin P. Creaser kindly identified the crayfishes. Ten specimens from each locality were studied. Under a binocular microscope the exterior was carefully examined for ectoparasites and the whole body was then picked apart and examined piecemeal. Ostracods, discodrilids, rotifers, and nematodes were found as follows:

#### Ostracoda:

681 *Entocythere cambaria* Marshall on the gills of 21 crayfishes.

#### Discodrilidae:

357 *Branchiobdella americana* Pierantoni about the bases of the antennae and other appendages and in the gill chambers of 11 crayfishes.

175 *Bdellodrilus illuminatus* Moore in the gill chambers of 9 crayfishes.

#### Nematoda:

340 *Rhabditis cambari* n. sp. between the gill plates of 13 crayfishes.

#### Rotifera:

213 *Embata parasitica* Giglioli on the gills of 9 crayfishes.

The twelve male crayfishes examined all carried other animals, but only 83 per cent of the females were so infested. Probably the small size of some of the latter, rather than the sex, was associated with the absence of guests or parasites. This assumption is further supported by the fact that the group of larger crayfishes, those found in the streams near Charlotte, had more parasites per individual than the smallest group, those from the Durham reservoir, which contained the fewest parasites per individual of the three groups examined.

The specimens of *Branchiobdella americana* found on the antennal bases were superficially different from those found in gill chambers. The

larger size and tougher skin of those living an ectoparasitic life was probably due to their environment.

Ameel (1932) has recently found the metacercariae of lung flukes (*Paragonimus*) in six species of crayfishes in Michigan. No trematodes were found by the writer.

***Rhabditis cambari* n. sp.**

Eunematoda, Rhabdiasaidae, Rhabditidae, Rhabditinae.

Host: *Cambarus acuminatus* Faxon and *C. blandingii* Harlan.

Types: 8692 and 8693, United States National Museum.

This parasite was found in the gill chamber of crayfishes. The cuticle is unstriated and without bristles. The male and female are about the

TABLE 1

PARASITES AND COMMENSALS ASSOCIATED WITH NORTH CAROLINA CRAYFISHES

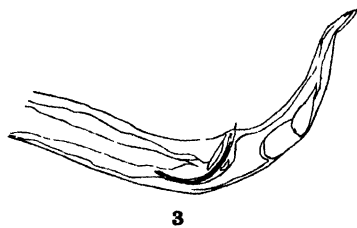
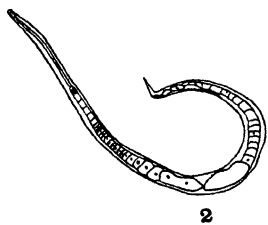
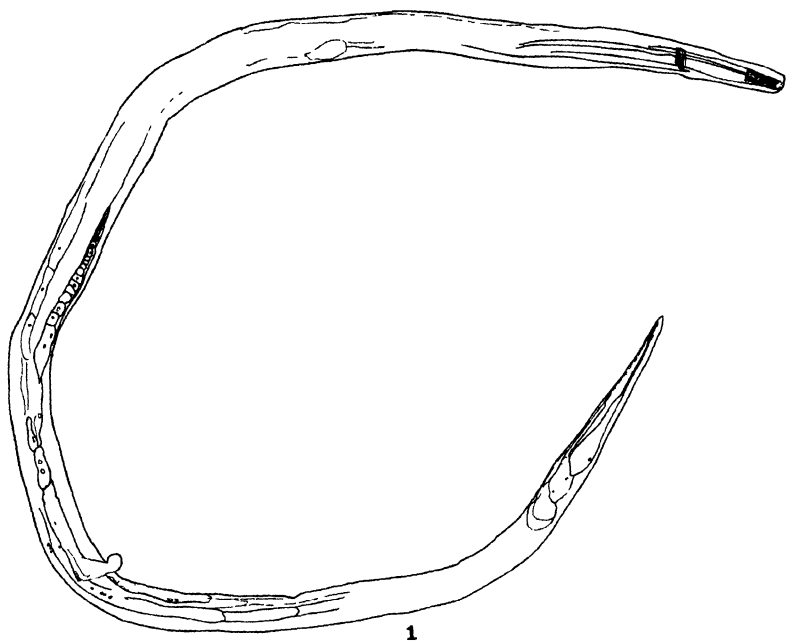
The first figure indicates percentage of crayfishes infested; the second, the number of parasites or commensals per individual.

LOCALITY AND SPECIES	NUMBER EXAMINED	SEX	ENTOCTYRHE CAMBARIA	BRANCHIOBELLA AMERICANA	EDELLEIDUS ILLUMINATUS	EMBATA PARASITICA	RHABDITIS CAMBARI
Durham Reservoir, <i>Cambarus acuminatus</i>	10	7 F. 3 M.	60 2.0	20 1.5		10 2.7	20 2.8
Hibbard's Pond, <i>Cambarus acuminatus</i>	10	5 F. 5 M.	60 22.1	90 34.2		10 0.4	30 14.3
Charlotte Streams, <i>Cambarus blandingii</i>	10	6 F. 4 M.	90 44.4		90 17.5	60 18.2	80 16.9

same size, averaging 0.77 mm. in length and 0.07 mm. in width. The body is fusiform and tapers toward the anterior end to two indistinct lip lobes. The esophagus extends through the anterior fifth of the body, bears internally three rows of small lateral teeth in the anterior half, and is without a bulb. Apparently there is a nerve ring around the esophagus. The anus is 0.015 mm. from the posterior extremity. A clear, bilobate, bladder-like gland is apparent near the posterior end with a duct which seems to run posteriorly to the outside. The vulva is a little posterior to the center of the body. The uterus has two horns extending anteriorly and posteriorly from the vulva. It contains eggs



PLATE 3



ranging in size from about one-fourth to three-fourths of the diameter of the body. The testis is single and in the posterior third of the body. The two slender, curved spicules are about equal in size and average 0.11 mm. in length. The presence of a gubernaculum is doubtful.

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#### EXPLANATION OF PLATE 3

##### REABDITIS CAMBARI

Fig. 1. Lateral view of female, showing uterus slightly prolapsed through vulva.

Fig. 2. Lateral view of mature female.

Fig. 3. Posterior end of male.



# A DEVELOPMENTAL STUDY OF DUMORTIERA HIRSUTA (SW.) NEES.\*

By PAUL M. PATTERSON

## PLATES 4-8

### INTRODUCTION

Fruiting *Dumortiera hirsuta* was found on the bank of a small stream in a ravine with a northern exposure, in a beech-oak woods near Columbia, S. C. Since no detailed study has been made of the development and cytology of this form, and since this genus is typically a tropical and sub-tropical one and therefore inaccessible to most botanists, it was thought that a study of the development of *Dumortiera hirsuta* would be of interest, both for itself and for comparison with other members of the *Marchantiaceae*.

Good descriptions of the gross morphology of *Dumortiera* including the structure of the thallus, surface, rhizoids, ventral scales and receptacles are available from several sources: e.g., Nees von Esenbeck (18), Leitgeb (37), Goebel (24), Engler and Prantl (15), Ernst (17), and others. A good account of the geographical distribution of this genus is given by Evans (20) and Herzog (32).

Evans (20) lists all the *Dumortieras* described under two species: *D. hirsuta* (Sw.) Nees, and *D. nepalensis* (Tayl.) Nees. This arrangement is given also by Evans (19) in his description of *Dumortiera* in the North American Flora where *D. trichocephala* Hook, *D. velutina* Schiffn., and *D. calcicola* Campbell are considered synonyms of *D. nepalensis* (Tayl.) Nees. Campbell (9) describes the development of antheridia in *D. velutina*, and of archegonia in *D. trichocephala* as being typically marchantiaceous. The earlier development in the embryogeny of *D. velutina* is given and a general account of the later development is discussed for both species. Ernst (16 and 17) made a study of the relative number of androgynous receptacles from several collections of *Dumortiera velutina* and *D. trichocephala*, showing that the former is chiefly dioecious and the latter monoecious. Later he reports in much detail

\* Botanical Contribution from the Johns Hopkins University No. 121.

on the relative number of androgynous receptacles, and the relative proportion of male, female, and bisexual receptacles on the same thallus in these collections. He comes to the conclusion that the androgynous inflorescence here is a secondary or recent development: that is, dioecious species are primitive among the *Marchantiaceae*, monoecious forms are derived, and the androgynous receptacles are the culmination of this tendency. Heitz (31) puts the number of chromosomes for *D. hirsuta* at nine.

All of the *Dumortiera hirsuta* material used in this study was collected from the one station near Columbia mentioned above, except for one lot showing various stages of antheridial and archegonial development, which was collected in Jamaica, B. W. I., July 1932. *Dumortiera hirsuta* was found in two other localities about Columbia but all these plants have been sterile. Most of this material was fixed in either formal-acetic-alcohol, or in chrom-acetic osmic mixtures. The haematoxylin and various anilines, singly and in combination, were used for staining. The method that proved most efficient and convenient for general developmental stages was over-staining the sections in saffranin and reducing the saffranin to the desired point with Bismarck brown.

The development of *D. hirsuta* in Columbia is seasonal, whereas in Jamaica the various organs are initiated apparently any time of the year. A typical schedule of development for *D. hirsuta* at the Columbia station recorded in 1932-33 is as follows:

- August 11-19. All male receptacles were initiated.
- September 5-12. All female receptacles appeared.
- September 10-20. Spermatogenesis occurred in most antheridia.
- September 15-25. Most archegonia matured.
- September 25 to October 5. Majority of fertilizations occurred.
- October 5 to October 15. Young embryos have formed.
- October 15 to November 3. Foot and archesporium differentiated.
- November and December. Growth of sporophyte, formation of spore mother cells, and differentiation of elaters.
- December 20 to January 20. Growth of capsules and spore mother cells completed. Antheridial branches withered.
- January 26 to February 4. Meiosis occurred.
- February. Maturation of spores occurred, elaters, and cells of capsule wall thickened.
- March. Blackening of capsules, fertile thalli dying, and receptacles elongated.
- April. Setae elongating and spores liberated.

In dry years this schedule is somewhat modified.

## THE FORMATION OF THE RECEPTACLES AND SEX ORGANS

The thallus of *Dumortiera hirsuta* grows by a wedge-shaped four sided apical cell located in a notch at the end of the branch. The notch is the result of the more rapid growth of the lateral tissue derived from the apical cell than of that immediately behind it. In a sagittal section also the apical cell occupies a notch and is nearer the ventral than the dorsal surface because the dorsal region grows more rapidly than the ventral and projects somewhat over the latter. Ventral scales are initiated on either side of the median sagittal plane. These scales overlap each other and the proximal ones project over the apex of the thallus as is common in the *Marchantiaceae* (Douin 12). The ventral scales often consist of a short limb with two wings, each covering a rhizoidal bundle. There is one main rhizoidal groove and several smaller lateral ones at first at the branch apex, though the ventral scales disappear early and both the plain and the peg rhizoids then lie free on the smooth ventral surface a few millimeters from the growing tip.

*The Male Receptacle.* The male receptacle commences as a slight mound of tissue pointing diagonally downward. The products of the apical cell, instead of maturing rapidly, remain actively meristematic and the apical cell becomes obscured. Soon a short downward pointing arm of rapidly growing tissue has been formed (fig. 106). The proximal half now commences to thicken up thus forming the primordium of the head, at the distal end of which are now distinguished several apical cells (fig. 107). Soon after the commencement of the male receptacle, all of the tissue at the base of the receptacle is morphologically ventral—witness the appearance of ventral scales on the upper side of the primordium in figure 106.

With the marking out of the apical cells, the proximal segments become the dorsal surface, the distal ones forming the ventral surface of the head. The stalk with its two ventral grooves is thus morphologically a ventral process. The size of the head is increased at first by divisions of all of the cells of the surface in addition to the tissue added by the apical cells. More rapid growth on the lower side of the stalk pushes the receptacle upward carrying it beyond the dorsal lip of the thallus. Sometimes growth is so much more rapid on the lower side that the head region is distinctly formed there before it has commenced to differentiate on the upper side. Antheridia always appear first on the lower side. The tissue at the top of the stalk just under the head remains slightly meristematic, gradually raising the maturing antheridial receptacles slightly above the thallus.

*The Antheridium.* When the male receptacle has attained a diameter of 0.6 to 0.8 millimeter, it commences to form antheridia. These are formed in radial series from the dorsal segments of each one of the apical cells. Antheridia are formed from about 20 to 25 apical cells each giving rise to a succession of 3 to 5 antheridia. As in the other marchantiaceous genera, the antheridia become sunken in pits on the lateral and dorsal surface accompanied by several (up to four) one to two celled glandular paraphyses. The terminal portions of these paraphyses are sometimes swollen. They persist only during the younger stages of the antheridia.

The antheridia become displaced as they mature so that in a section parallel to the dorsal surface of the receptacle the hundred or more antheridia are not in radiating rows but are considerably displaced, differing in this respect from *Marchantia* as Campbell (9) has pointed out in describing *D. velutina*. The antheridial initial is seen as a cell standing free from those surrounding it (fig. 1), recognizable within 2 or 3 cells of the growing point. It does not protrude above the general level of the thallus as in *Marchantia* (Strasburger 52). The initial now divides to a series of four cells in a row (figs. 2, 3, and 4), and is soon overgrown by the adjacent tissue of the thallus thus leaving the antheridium in a pit (figs. 2 and 3), as in *Marchantia* (Durand 14). Anticlinal walls appear and usually 2 to 4 central cells have divided when the antheridium is 6 cells long leaving only the stalk cell and terminal cells undivided. Figure 9 shows the cross-section of an initial consisting of a single row or cells; figure 10, the cross-section of a central cell of an antheridial initial showing the first longitudinal anticlinal division. Figure 11 shows that the next anticline is at right angles to the first dividing the central portion of the filament into four cells. Figure 5 shows an antheridium 8 cells long with the central 3 divided by anticlinal walls. Here the stalk and the body of the antheridium have already become differentiated. In figure 6 the anticlines have divided the cells at the tip of the antheridium whereas in figure 7 the anticlines have gone through the antheridial stalk, though this does not usually occur until considerably later. In figure 6 anticlines separating the inner spermatogenous tissue from the antheridial wall have commenced at the lower portion of the antheridium proper and are extending towards the cap cells. In figures 7 and 12 the spermatogenous cells are seen to have formed. Figure 8 shows that the spermatogenous tissue has commenced to divide further and figure 13 shows a cross-section at a slightly later stage. The antheridium is increasing in size all the while but does not

keep pace with the increase in spermatogenous cells, resulting thus in smaller cells as division continues. Figure 14 shows a nearly mature antheridium with its cap cells protruding into an apiculate tip as in *Riccia*, *Targonia*, or *Fimbriaria*. The stalk is two cells in section and is long and slender, differing from *Fimbriaria* and *Marchantia* in this respect, but similar to *Targonia* as figured by McFadden (39). When mature, an antheridium contains within wide limits, about fifty thousand antherozoids or about five million per male receptacle. This estimate for the antheridium is somewhat smaller than the figures given by Johnson (36) for the antheridium of *Monoclea*. The wall cells of the antheridia are turgid throughout development. The antheridia open by a terminal rupture, but no details here were observed.

The formation of a row of 3 or usually 4 cells in the antheridial filament agrees with Campbell (9) for *D. velutina*, Strasburger (52) for *Marchantia polymorpha*, Dupler (13) and Haupt (28) for *Reboulia hemisphaerica*, and McFadden (39) for *Targonia* though it may be 4 or 5 cells long before anticlines commence in *Monoclea* (Johnson 35) and *Fimbriaria* (Campbell 10). Anticlinal walls commence at the center of the antheridial filament, differing from *Reboulia* in this respect (Haupt 28). The periclinals to form the central cells begin in the lower portion of the antheridium, corresponding to *Marchantia* (Durant 14), and differing in this respect from *Reboulia* (Haupt 28). The separation of the central cells is typically marchantiaceous, the lowest row remaining sterile and forming the basal wall of the antheridium.

*Spermatogenesis.* The development of the spermatogenous tissue progresses unevenly in regard to cell division. Certain blocks of cells undergo mitosis simultaneously, though these regions do not necessarily coincide with an original spermatogenous cell or group of cells, although at times this appears to be true.

The last or diagonal division is probably the tenth or eleventh mitosis in the antheridium after the spermatogenous tissue has been differentiated. It commences earlier in some parts of the antheridium than in others though the developmental stages of the androcyte, to use Allen's (1) terminology, to form the antherozoids, are remarkably uniform throughout the antheridium. All of the antheridia of one receptacle produce antherozoids within a short time of each other, those at the periphery of the receptacle, being younger, maturing last.

The best preparations here were obtained with Haidenhain's iron haematoxylin stain after fixation with Flemming's strong solution. The formation of the androcyte is preceded by the diagonal division. This

division was followed in detail. Ten chromosomes were seen in the last division. No centrosomes here or in the earlier divisions of the spermatogenous tissue were seen. The other features of the last division here correspond closely to those observed in other *Bryophyta* [Ikeno (33); Wilson (56); Woodburn (57, 58, 59); Allen (1); Sharp (49); etc.].

The nucleolus now divides as though in preparation for another division. About this time, or sometime earlier, a granule appears in the cytoplasm some distance from the nucleus (figs. 49 and 50). It is larger and somewhat more diffuse than it later appears when it has moved to the proximity of the nucleus. This is the blepharoplast. The blepharoplast is identified with the centrosome in the case of *Marchantia* by Ikeno (33), of *Fegatella* by Bolleter (8), of *Pellia* by Wilson (56), of *Polytrichum* by Allen (2) and Walker (55), and of *Blasia* by Sharp (49). The blepharoplast is considered of possible centrosomic origin in *Plagiochila* (Johnson 36). It originates in the cytoplasm in *Riccia* according to Black (6), and Lewis (38) considers it a centrosome-like structure. Woodburn (57, 58, 59) considers the blepharoplast to be of cytoplasmic origin in all the forms he investigated: *Mnium*, *Asterella*, *Blasia*, *Porella*, *Marchantia*, and *Fegatella*. Wilson reports that the blepharoplast arises from a fragment of the nucleolus that passes to the cytoplasm in *Mnium hornum* and *Atrichum undulatum* and Bagchee (4) thinks it is of nuclear (chromatin) origin in *Anthoceros*.

From the work done on spermatogenesis among the bryophytes thus far, it seems safe to conclude that a centrosome-like body is often present in the last division of the spermatogenous tissue and the centrosome becomes transformed to a blepharoplast in the maturing androcyte. We might look upon cases where a blepharoplast still appears in the cytoplasm after the penultimate division, as an example of the retention by the plant for a special function in the spermatozooids of a body (the centrosome) which has ceased to exist in the vegetative cells.

The nucleolus sometimes cuts off two or three granules of varying sizes. These may evidently be formed in rapid succession, as they are sometimes seen to lie in a short chain. At this time the blepharoplast is already present in the cytoplasm. One of the larger nucleolar granules remains as the nucleolus of the maturing antherozoids, and soon shows an areola. The other fragments disappear from the nucleus eventually, but whether they degenerate or pass to the cytoplasm is uncertain. A "percnosome"-like body (Allen 2) is seen in the young androcyte. It is

a hyaline, spherical body with a granule at its center. This structure disappears in the maturing androcyte.

The story of the formation of the androcyte is that typical for bryophytes. The blepharoplast lies near the nucleus. It elongates at first to form a thick rod, but later becomes a long thin thread in intimate connection with the elongating nucleus and projects a considerable distance anteriorly (figs. 52-59). Figure 58 shows a nucleus so displaced that the extent of the blepharoplast can be seen. The nucleus after elongation has set in becomes somewhat flattened in a plane parallel to the diagonal wall mentioned, and then curved. By continued elongation and curving, the nucleus together with the blepharoplast soon gives the young antherozoid the appearance of a ring. It is really a watch-spring-like coil of about  $1\frac{1}{4}$  turns. About this time the nucleus stains so deeply that it remains black with haematoxylin when destaining has gone far enough to make the other structures of the cell quite distinguishable. As the nucleus elongates further it becomes a cylindrical body tapering toward both ends. Preparations of such stages that have been destained until translucent show the nucleolus with the originally round areola much elongated. The nucleolus was not seen in mature antherozoids.

The formation of the two cilia was not seen. They can be observed after they have become rather long (fig. 59). Figures 60-64 were drawn from smears made from teased antheridia, and show stages in the rolling back of the cytoplasm to form the vesicle at the posterior end of the antherozoid. The cilia are long; one is attached at the anterior tip of the antherozoid, the other, somewhat posteriorly. Steil (51) found the cilia to be attached thus in *Riccardia*. The cilia are 3 to 4 times as long as the body of the antherozoid, the posterior cilium being longest. The following are usual dimensions: body of antherozoid 13 microns in length, anterior cilium 32-37 microns, and the posterior cilium 46-55 microns. Figure 64 shows the antherozoid dried and stained in a position characteristic of its active form.

By the time the sperm nucleus has elongated to form a ring the walls of the androcyte mother cells have become difficult to demonstrate. In the mature antheridium all cell walls of the spermatogenous tissue have disappeared. The androcytes lie closely packed in pairs in a mucilaginous medium. Campbell (9) reports the "sperm cells are not so evidently in pairs" in *D. velutina*. Upon ejection of the antheridial contents into water the cilia of the androcytes vibrate violently, freeing themselves from each other and from the mucilaginous matrix.

*The Female Receptacle.* Both male and female receptacles are borne on the same thallus, all the plants observed being monoecious. Female receptacles are initiated in a similar manner to the male receptacles. They become recognizable when the apical tissue of the head commences to form a narrow, ventral scale-free meristematic zone (fig. 108). Archegonia commence to be initiated when the receptacle is about 0.7 of a millimeter in diameter. These are formed in acropetal succession from each of the 7-15 growing points differentiated on each head. Campbell (9) reports 6-7 "apices" for *D. trichocephala*. There are usually three archegonia formed from each apical cell. These are formed in rapid succession in one radial row on each growing point as indicated in figure 15.

*The Archegonium.* The development of the archegonia is typically marchantiaceous, and follows the development given by Campbell (9) for *D. trichocephala* closely. A surface cell of the lower side of the receptacle divides to form a stalk and an archegonium initial (figs. 16 and 17). This initial protrudes beyond the level of the thallus and often is somewhat larger than the stalk, as was shown by Strasburger (52) for *Marchantia*. The stalk cell may be divided by a partial or usually complete longitudinal wall before the archegonium initial has divided (fig. 17). The archegonium initial is divided by the usual three longitudinal walls (figs. 15, 18, and 26). A transverse wall then appears separating the cap cell from the central cell (fig. 21). The central cell then divides in the usual way to form the egg cell, the ventral canal cell, and the neck canal cells (figs. 15, 20, 27 and 28). Figure 25 shows a transverse section through an archegonium at such a stage as figure 22 after a division has occurred in a wall cell. The ventral canal cell disintegrates long before the neck canal cells, forming a dark mucilaginous cap on top of the egg. Although mitotic figures were seen only in the divisions of the neck and the neck canal cells, the origin of the various cells of the archegonium can be identified rather certainly by the slight difference in cell size, cell wall placement, and density of cell contents. The neck of the archegonium at maturity is usually 6 cells in circumference (fig. 23) occasionally 7 (fig. 24). Six is the typical number for the *Marchantiaceae* (Janczewski 34). Johnson (35) found that the necks of the archegonia in *Monoclea* show 5-8 cells in cross section. The neck of the archegonium elongates as maturity is reached until it is 20-25 cells long, those nearer the venter being short and increasing in length as the apex is approached. The mature archegonium is somewhat larger than that reported for *D. trichocephala* by Campbell (9).



and is longer than in *Marchantia* (Durand 14) though not as long as that of *Fegatella* (Janczewski 34). At maturity the neck bends outward and upward around the edge of the receptacle. Thus the tips of the archegonia lie in the angle just below the upper surface of the head and next to the outwardly protruding ventral scales. An unopened tip in optical section is shown in figure 30 and an opened unfertilized archegonium in fig. 31. A surface view of an opened fresh archegonium is given in figure 32, showing 5 turgid cap cells which have slightly thickened outer walls and are separated somewhat from each other and from the neck cells. Soon after opening, the neck canal cells degenerate completely. The egg lies surrounded by the mucilaginous remains of the ventral canal cell. During the development of the archegonium the wall of the venter has become 2 and 3 cells in thickness in which it differs from *Marchantia*, *Preissia*, and *Lunularia*, but resembles *Plagiochasma* and *Reboulia* (Janczewski 34), and *Monoclea* (Johnson 35). The ripe egg normally fills the venter. The nucleus and its nucleolus are large. There is no receptive spot.

Occasionally abnormal archegonia are seen. One such abnormality is figured (fig. 29) showing the egg, ventral canal cell, and neck canal cells in duplicate. Another abnormal archegonium lacked a venter, a neck canal cell becoming apparently the egg cell. Still another variation was observed where a single egg and ventral canal cell were followed by a double series of neck canal cells.

*The Involucre.* Usually after the formation of the third archegonium from any one growing point of a receptacle the apical cell becomes inactive as such and is carried up by a ring of tissue (all of the marginal cells of which are two sided in radial section) surrounding the archegonial group on the axial and lateral sides. This is the commencement of the involucre. Occasionally two involucre are initiated, the inner one growing up about a single archegonium. There is no perianth developed. The distal side of the archegonial group is usually bordered at first by the overhanging edge of the receptacle. The involucre is not the result of fertilization and in this respect is similar to that of *Monoclea* (Johnson 35). At the time of fertilization the involucre is about the same height as the venter of an archegonium. With further growth of the involucre its abaxial portion differentiates from the ventral tissue of the edge of the receptacle. Growth of the whole female receptacle now takes place on all radii where fertilization has occurred. The opening of the involucre narrows and by the time sporogenous cells in the embryo have become elongated and separated from each other,

the involucre has become a closed pointed sac surrounding the enlarging venter of the archegonium and closed about the neck end of the latter. This involucre differs from that of *Conocephalum* as described by Graham (25) where more rapid growth on the axial side of the involucre (in the latter) brings the point of closure nearly opposite the foot of the sporogonium. The growth of the involucre is more rapid than the enlarging embryo, thus leaving a considerable space between the two. The mature involucre bears numerous bristly hairs.

*Bisexual Receptacles.* Bisexual receptacles were first reported in the *Marchantiaceae* in *Dumortiera irrigua* by Taylor (54). Such receptacles have since been observed in various other *Marchantiaceae* as has been noted in a review by Haupt (30). A detailed study of these bisexual receptacles in *D. trichocephala* and *D. velutina* was made by Ernst (16, 17). He found them common in the former species but rare in the latter. Bisexual receptacles were found frequently in the Jamaica collection where about half of such receptacles were typically male, the other half female. However, bisexual receptacles vary from predominantly male to predominantly female, the various radii being either wholly male or female. There is no alternation of radii of different sexes, those of the same sex always adjoining each other. Bisexual receptacles occur rarely at Columbia, only two being found out of the hundreds of receptacles observed.

#### THE DEVELOPMENT OF THE SPOROPHYTE

*Fertilization.* No observations were made in the living plant on fertilization. After the entrance of the sperm, the male nucleus enlarges considerably becoming elongated at first. This agrees with the structure of the male nucleus of *Sphaerocarpus* according to Rickett (47). In *Riccardia*, the sperm nucleus becomes longer and more attenuate during and after penetration of the egg as shown by Showalter (50). The nucleolus of the male soon becomes visible and the nucleus rounds up. Both the male nucleus and its nucleolus are considerably smaller than the corresponding ones of the female. A membrane soon surrounds the egg (fig. 36). Judging from the frequency of the appearance of two separate nuclei in the egg, it is assumed that there is a considerable period after the entrance of the antherozoid before the fusion of the two nuclei takes place. The male nucleus approaches the female nucleus (figs. 35 and 36), and gradually merges with it (fig. 37). Here a distinction between the chromatin net of the egg and that of the sperm can yet be seen. At a slightly later period the two reticuli are

indistinguishable and the nucleoli are in the process of fusing (fig. 38). The chromatin was not seen to emerge from the reticulate phase as described in *Corsinia* and *Fegatella* by Meyer (41, 42), in *Sphaerocarpus* by Rickett (47), in *Riccia* by Black (6) and Garber (23), in *Preissia* by Graham (26), and in *Riccardia* by Showalter (50). The fertilized egg remains undivided for a considerable period, during which the venter of the archegonium enlarges and becomes four or five layers thick (fig. 33).

*The Embryo.* Fertilization may take place in all of the archegonia of any involucre group and embryonic development begin. One embryo, however, soon takes the lead, the suppressed embryos rarely developing beyond 2-8 cells. The fertilized egg elongates before the first division, the mitotic spindle lies in its axis and the cell divides to two usually approximately equal halves by a wall transverse to the longitudinal axis of the venter (fig. 39). Occasionally the epibasal cell may be larger as in *D. velutina* (Campbell 9) and the first wall may often also be somewhat oblique. The next wall may be transverse also, forming thus an embryo of three cells in a row as reported for *D. velutina* (fig. 42). In other cases the next walls may be perpendicular to the first, dividing the embryo into quadrants (fig. 40). In the quadrants the next walls are vertical thus dividing the embryo into octants (fig. 41). In the filamentous embryo (fig. 42) are shown vertical walls after several have appeared, whereas in the 8-celled embryo shown in fig. 43, the vertical walls have appeared in a plane parallel to the section, and the distal region has commenced to subdivide. The octant type of embryo found here occurs also in *Marchantia polymorpha* (Durand 14), *M. domingensis* (Anderson 3) *Riccia* and *Targonia* (Campbell 10), *Preissia* (Haupt 30), *Riccio-carpus* (Garber 23), though in the latter it may sometimes form a row of cells, and *Oxymitra* (Sealey 48). Meyer (41) considers the quadrant type to be more advanced than the "filamentous" one. Admitting this then, *Dumortiera* may be considered intermediate in this respect between *Marchantia* on the one hand and *Conocephalum* or *Reboulia* on the other. Anticlinal and periclinal walls now come in irregularly forming a sphere of tissue (figs. 44-48), as in *Marchantia* or *Oxymitra* (Sealey 48). The capsule wall and primary archesporial cells are delimited by longitudinal periclinal walls (fig. 44). The venter of the archegonium increases considerably in size during the early development of the embryo. Its wall becomes 8-10 cells in thickness by the time the sporogenous tissue begins to stain differentially—and thus differs in this respect from *Marchantia* (Durand 14). Further enlargement of the venter or calyptra to accommodate the growing sporogonium is not accompanied by

further growth in thickness of its wall. The original quadrant walls are indicated diagrammatically in figures 45 and 47. Figure 48 shows a longitudinal section of an embryo in which the sporogenous tissue is being differentiated. It is possible that the capsular region of the embryo is formed from the epibasal cell of the embryo, and the foot and stalk regions, which never become markedly differentiated, are produced by the hypobasal cell. The sporogenous tissue stains more deeply and becomes distinguishable from the wall cells of the capsule while the embryo is still spherical (fig. 48); the basal portion of the embryo embedded in the floor of the venter becomes the haustorial zone. The sporogenous cells continue to grow and to divide, chiefly by longitudinal walls. Thus as the embryo enlarges the sporogenous cells become elongated in the direction of the axis of the capsule (figs. 109 and 110). No cap or extra layer of sterile cells remains at the apex of the capsule as in *D. trichocephala* (Campbell 9), *Marchantia chenopoda* (McNaught 40), *M. domingnensis* (Anderson 3), or *Fegatella* (Meyer 42).

The cells within the capsule continue to elongate and later separate from each other. The young elaters and definitive sporogenous cells are not as yet different in appearance. The cells at the center of the capsule are always longer and narrower than those near the periphery (figs. 65-68). These sporogenous cells are longer than in *Marchantia*. The nucleus now elongates in the sporogenous cells, and divides (figs. 70-73). Cross-walls were not demonstrated at this stage (fig. 74). Later, the cytoplasm increases about each nucleus giving the hitherto spindle-shaped cell a nodular appearance (fig. 75). After the third nuclear division, cross walls become clear and thus a chain of 8 (or perhaps more) spore mother cells is finally formed (figs. 82 and 111). The cells destined to form elaters, on the contrary, continue to elongate greatly (fig. 69), but their nuclei never divide. Though the spore mother cells hang together in chains at first they are readily separable before divisions in these cells are completed. As divisions of the nucleus of the sporogenous cell take place, spore mother cells are formed in the middle region of the original cell. This leaves the two more or less attenuate ends of each sporogenous cell attached to the terminal spore mother cell of each series (figs. 80 and 82). The row of spore mother cells shown in figure 82 was formed from a sporogenous cell such as is shown in figure 65, while the terminal spore mother cell such as that shown in figure 80 was formed from a cell as shown in figure 68. The process referred to is finally digested and the terminal spore mother cell rounds up to resemble the other spore mother cells of the chain. A single layer of

sporogenous tissue next to the capsule wall does not develop into spores and elaters but disintegrates, forming a sort of tapetal addition to the mucilaginous content of the capsule, an apparently unusual condition in the liverworts. The spore mother cells later separate and round up though they still lie in rows (fig. 111). When first formed they measure 11 to 13 microns in diameter and their nuclei are about 7.6 microns (figs. 76 and 77). The spore mother cells continue to increase in size (figs. 78 and 79) and the elaters continue to elongate. The mature mother cells are approximately twice their original size (26–27 microns in diameter, with nuclei 13 microns in diameter) (fig. 81); while the elaters have attained a length of about 700 microns.

This increase in size is at the expense of the dense cytoplasm of the young spore mother cell, the mature spore mother cell being rather vacuolate here as it is in *Corsinia* (Meyer 41). The ripe spore mother cell is spherical, as is typical of the *Marchantiaceae*. Dense mucilage fills the interstices between the spore mother cells and elaters. This becomes thinner and finally disappears during the maturation of the spores.

*Meiosis.* Various fixatives were tried for this stage of development, those proving most efficient were Flemming's strong, and the Chicago modification of Flemming's as listed by Chamberlain (11). The sporangial wall at the time of meiosis is an effective barrier to the penetration of fixatives of the chromic acid series, consequently, to facilitate fixation, one-fourth to one-third the diameter of each sporogonium was cut away. Contrary to what might be expected few spore mother cells were lost. Even when the spore mother cells were thus exposed, the fixation was often incomplete and some plasmolysis was evident in the center of the sporogenous mass. The stains most effective after fixation were either safranin alone or in combination with gentian violet or Bismarck brown, or Flemming's triple stain. Varying schedules for Heidenhain's haematoxylin were tried but, as Blair (?) also found true in the case of *Reboulia*, good differentiation was not obtained. Sections were cut from 3–5 microns in thickness.

There was no observed periodicity in the mitoses. Lots were fixed at various hours of the day and night but metaphases appeared in about the same proportion (about 10%) no matter when the sporogonia were fixed.

No evidence could be found in the fixed material of amoeboid movement of the spore mother cells noted for instance by Haupt (29) in *Reboulia* and Moore (44a) in *Pallavicinia*. The spore mother cells of

*Dumortiera* are round and their nuclei are round and turgid unless shrunk by poor fixation. No accurate data were obtained regarding the time involved in the reduction division. It must be very short since on January 30, 1933, few tetrads were formed, and by February 4, very few prophase were encountered. After prophase is well advanced the process must be very rapid since there exists no resting period between the two divisions and figures of both divisions are sometimes seen in the same capsule.

During the ripening of the spore mother cell, kinoplasmic plates make their appearance (fig. 81). They were seen only in the meiotic phase of *Dumortiera*. They seem to be characteristic of this phase in a number of forms: noted in *Pallavicinia* by Moore (44a), *Fegatella* by Meyer (42), and in *Reboulia* by Blair (?). In *Dumortiera* Gram's stain revealed elliptical bodies in these plates (fig. 91). They were never seen in material stained with saffranin alone or in combination. The ripe spore mother cell is surrounded by a wall about 0.5 micron in thickness. The prophase, judging from the frequency of its appearance (about 50% of the capsules), occupies a considerable time. The first indication of its commencement is the enlargement of the nucleus and the rearrangement of the linin to form a spireme. This spireme is poor in staining substances as was noted by Farmer in *Fossombronia*. Spiremes have been seen by various workers in this field: Moore found a definite double spireme in *Pallavicinia* which after contraction broke up to form chromosomes; Meyer (41) found a synaptic spireme in *Corsinia*; Florin (22) found the same condition in *Chiloscyphus*; Beer (5) found a spireme in *Riccia*, Meyer (42) in *Fegatella*, and Graham (26) in *Preissia*. In *Dumortiera* the spireme appears as an extremely fine, highly convoluted thread, apparently continuous (fig. 83).

The spireme then undergoes synapsis, contracting toward one side of the nucleus (fig. 84). The nucleolus is usually located at one edge of the knot, and partially embedded in it. The release of the spireme from synzezeis soon takes place, and it again fills the whole nucleus (fig. 85). The chromatin granules are becoming more conspicuous. The spireme begins to contract to the periphery of the nucleus against its wall (figs. 86 and 87) as was noted in *Fossombronia* by Farmer (21). Here the chromatin granules become fewer and larger and most are grouped in pairs. With the retreat of the spireme to the nuclear wall, a continuous spireme seems to give way to irregular wefts on which are disposed chromatin granules. Blair (?) noted a similar character in the post-synaptic spireme. The paired chromatin granules give the only evi-

dence of a possibly double spireme. These chromomeres seem to fuse until paired chromosomes are found at the periphery of the nucleus. Occasionally one or two of the pairs of chromosomes are formed in advance of the others.

Farmer (21) noticed that the chromatin granules in *Fossombronina* become aggregated along special thickened areas of a thread, and there condense to form chromosomes. Van Hook (60) reports that the linin is dissolved, the chromosomes then appearing; while Meyer (41) reports that the chromatin bodies of the nucleus of *Corsinia* fused to form chromosomes.

The nucleolus which has been visible throughout this process now rapidly disappears or at least loses its staining power and cannot be seen by the time the chromosomes are formed. The nucleolus may divide in two before the chromosomes have been formed, though it usually disappears before dividing. The nucleolus here, as well as in other phases of the plant, consists of a hyaline center surrounded by a deeply staining outer region giving it in optical section the appearance of a ring. No connection between any nuclear material and the nucleolus was observed. The nucleolus shows no signs of a fragmentation such as was noted by Farmer (21) and Blair (7) nor of a loss of chromatin or other substances. It seems to disappear rapidly, as faintly staining nucleoli are rarely seen.

The nuclear wall now disappears and a spindle is formed. The origin of the spindle is not clear though it seems to be formed within the nucleus, with the spindle fibers first appearing at the poles and later becoming visible across the equatorial zone. The axis of the spindle is perpendicular to the kinoplasmic plates and its poles push into the plates but have no organic connection with them. Moore (44a) says that the spindle originates from these polar caps in *Pallavicinia*. The origin according to Blair (7) is doubtful in *Reboulia* but the completed spindle projects into the cytoplasm. In *Dumortiera* these kinoplasmic plates have started to split in two when the spindle is formed and by anaphase of the heterotypic division these halves have often become separated. The poles of the spindle then lie between the separating half plates. Bivalent chromosomes arrange themselves on the equator of these spindles. There seem to be ten such pairs. Sometimes these dyads display a tetrad character as noted in *Fossombronina* by Farmer (21) and *Chiloscyphus* by Florin (22). That this did not appear to be a constant feature in *Dumortiera* is probably the result of the difficulty of accurate stain differentiation for such small chromosomes. The

heterotypic chromosomes in material fixed with the Chamberlain's modification of Flemming's fluid were strikingly larger (figs. 89 and 90) than the univalent chromosomes of the next or homeotypic division though the chromosomes of both divisions appeared of the same size in Flemming fixed material.

During metaphase the chromosomes of the dyads pull apart. They seem to be extremely viscous, threading out along the line of separation to such an extent that in some cases the bodies of the chromosomes were indistinguishable from the zone of separation, the two separating chromosomes appearing as an elongated thread thinning in the middle (fig. 92). The chromatin later pulls apart and condenses into typical chromosomes by the time anaphase is well advanced (fig. 93).

Figure 93 shows ten chromosomes in the lower group and nine in the upper. The tenth is not drawn in the latter but can be seen in the slide to lie under the chromosomal group. A pair of small chromosomes is here seen to lie in advance of the rest. These were often seen here. At times they had nearly reached the poles before the other chromosomes had fully separated. A count of ten can rarely be had in a polar view of a metaphasic plate on this account. This behavior of this small chromosome was not seen at any other cell division in the plant.

The manner of separation of the dyads is undetermined. The chromosomes become compacted together at telophase (fig. 96), the chromatin merging together but soon separating into a number of granules distributed on irregular wefts of linin. No spireme was seen to form in preparation for the homeotypic division. The nucleus of figure 94 corresponds to the phase seen in figure 87 except that the chromomeres are not in pairs in figure 94. Each nucleus is now about half the size of the previous prophasic nucleus. The spindle fibers now become more numerous and a clear-cut cell plate is formed across them (fig. 94). When this plate has gone half way across the cell, the spindle fibers become distorted and give place to a thready vacuolate cytoplasmic meshwork. No distinct phragmoplast can be seen, though under favorable conditions the cell plate, which has now become a thickened, reticulate cytoplasmic band, is seen extended across the protoplast (fig. 97). The nucleolus reappears and is often seen to have divided into two. These daughter nuclei now become extremely elongated in a plane parallel to the previous cell plate, with the separated kinoplasmic plates lying at their poles (fig. 97). The nucleoli now disappear, the homeotypic spindles appear and the chromatin granules condense to form chromosomes and arrange themselves on the equator of the



spindle. Thus there is no true resting stage between the two divisions. The homeotypic spindles are somewhat more slender than the heterotypic ones (fig. 98). They may be parallel or at right angles to each other. Meyer (42) pointed out that these spindles were always parallel in *Fegatella* and Moore (44a) reports them as being perpendicular in *Pallavicinia*.

The separation of the split haploid chromosomes is rapid, the chromosomes reaching the poles and quickly becoming disposed as granules upon the reticulum. The outline of the nucleus remains irregular for a considerable time due to the adajutting cytoplasmic vacuoles (fig. 99). The spindle fibers rapidly disappear with the formation of a cell plate and four spores are formed in the manner described above. The peripheries of these four protoplasts soon take on definite margins, and become separated from each other by narrow spaces. Each of these four protoplasts now commences to secrete a wall about itself while the original wall of the spore mother cell has begun to dissolve. The nuclei of the spores now acquire a typical appearance, though they are often somewhat irregular in shape. They are delimited by a well-defined nuclear wall and contain a granular reticulum and a deeply staining nucleolus. The kinoplasmic plates disappear and the cytoplasm becomes somewhat thinner as the spore walls are formed.

#### MATURATION OF SPORES, ELATERS AND CAPSULE

Immediately after the formation of the spore tetrads, each spore protoplast separates somewhat from its neighbors and is delimited by its own plasma membrane (fig. 99). This membrane is seen in the ripening spore as a dark line on the interior of the other walls, slightly thickened in various regions due to cytoplasmic connections with the body of the spore. The protoplast now secretes an exospore, which soon reaches a thickness of 0.5–0.6 microns (fig. 100). A brown epispor is then deposited upon the exospore. As a membrane the epispor is thin, and it is continuous over the local thickenings or papillae of the exospore (fig. 101). These papillae attain a length of 1.6–1.8 microns. On the side next to a sister spore the ends are often bent due to the presence of the adjoining spore. The ends of these blunt spines are typically notched, but may be irregular or simple. With the development of the epispor, the old spore mother cell wall is completely dissolved. The reserve food in the spores is of a soluble nature, the body of the spores in section being empty except for the nucleus, which is usually crowded to one side, and a few cytoplasmic strands branching through the body

of the spore. The nucleus is of medium size, having, when spherical, a diameter of about 5 microns. The spores hang together in tetrads for a long time (fig. 114). The ripe spore is about 30 microns long and 23 microns thick.

Normally spores are formed in tetrads, infrequently in diads, and rarely in triads.

By the time the exospore commences to form, a hyaline double spiral band 2.7–3 microns wide is being laid down on the inside of the wall of the elater by a band of granular cytoplasm following a similar course. It is in this same manner that the thickenings are formed in the elaters of *Fimbriaria* according to Campbell (10). The spiral band soon becomes thick and brown in color. They are closely wound in the middle region of the elaters, but become lax towards the ends (fig. 114). They are usually united at the pointed ends of the elaters. The mature elaters contain no cytoplasm. The elaters are 7.2 microns wide and 0.6–0.7 millimeter in length. The formation of spore coats and elater thickenings reduces the mucilaginous contents of the capsule to a minimum.

In the wall cells of the capsule at about the time the elaters commence to thicken, short spiral, annular, or incomplete annular thickenings are deposited by special bands of granular cytoplasm (fig. 112). The capsular wall is one cell in thickness. The mature capsule not only has thickened wall cells but also has a layer one to two cells thick at the base of the capsule thickened by annulations or short spirals. Many of these cells are conical in shape, 50 microns wide at their bases and their pointed ends protruding 100–120 microns into the capsule. These are best seen in hand sections of the fresh capsule after washing away the spores and elaters. These cells evidently represent transition stages between tissue of the seta and the elaters.

During the maturing of the sporophyte, the stalk of the female receptacle elongates. The growth here is slow. By the time the capsules are blackening the stalks are about 3–10 millimeters long; when the spores are shed, the stalks range from two to three centimeters in length. Receptacles of some of the plants of *D. hirsuta* observed in Jamaica had stalks measuring up to six centimeters.

Stalks of elongating female receptacles were marked at millimeter intervals while still comparatively short, with carmine which had been ground into vaseline. Further elongation of the stalk did not separate these markings, but took place above the markings at the base of the head of the receptacle. Sections reveal a meristematic zone at the tip of the stalk, ventral to the tissue of the head.

When the capsule has matured, the seta elongates and pushes the capsule through the calyptra and involucre, rupturing these tissue layers. This elongation occupies from one to two days. The capsule appears black while the seta is whitish, though a few small, green chromatophores are present. The sporogonia protrude in a diagonally downward direction.

Just before elongation takes place, the stalk of the sporogonium is about 0.6 millimeter in length, whereas afterwards the stalk measures 1.8-2.2 millimeters, that is three or more times as long as before. The number of cells in the seta is the same before and after elongation, there being about 15 cells in the length of the seta. Before the elongation of the seta its cells are cubical or somewhat elongate and measure 30-60 microns; after elongation these cells become three or more times as long as before, measuring 100-180 microns. Thus the total elongation of the stalk is effected by the lengthening of the cells composing it. In this it agrees with the seta of *Monoclea* described by Johnson (35).

The wall of the capsule is composed of rows of cells radiating upward from the seta which give way to cells irregularly placed in the apical region. The wall cells are thickened chiefly by annulations. As the cells of the capsule wall dry and the water leaves the cell cavities, atmospheric pressure evidently flattens the cell wall about the annulations. At the apex of the capsule, where the cells are irregularly disposed, the resultant contraction of the cells causes them to pull apart in irregular groups. Most of these groups of cells are flicked off by the elaters that now commence to writhe as they dry. A few of the fragments remain attached to the side wall of the capsule. There is no annulus, and there is no lid or cap that comes off. The drying of the sporogonial wall which causes the contraction of the cells now produces tears in the capsular wall which follow the lines of least resistance, that is, they run between rows of cells down the sides of the capsule. There is no predictable line of weakness, the split may follow a straight line between two rows of cells, or it may pass from one row to another, or it may be rather irregular in its course. The drying and expanding mass of elaters now assist in splitting the capsular wall. There are usually five to eight fissures initiated, three to five of these may run at least three quarters of the way to the stalk. When the capsular wall is dry it forms a small shrivelled crown at the upper end of the seta and the spores and elaters are exposed as a spreading tuft. Many of the spores are flicked off during this initial phase by the elaters, some of them being sent to a distance of three millimeters. The spores are gradually

dislodged by hygroscopic activity of the elaters, wind and rain, and distributed by air and water currents.

Many of the elaters as they elongate in the developing capsule force their pointed ends between cells at the base of the capsule for a short distance. Presumably these elaters prevent the everted tuft of spores and elaters from falling away readily *en masse* from the sporogonium.

In dry air, the capsules open by the time they have become completely exserted from the involucre, often commencing to open before this. In a saturated atmosphere in a covered dish they do not open, but they open promptly when placed in less humid air.

O'Hanlon (45) estimates the number of spores for *Marchantia polymorpha* at not less than 300,000 per capsule. In *D. hirsuta*, counts of samples of well agitated dilutions from single capsules give much smaller figures: 30-40,000 spores for small capsules and 75-85,000 for large ones.

#### GERMINATION OF SPORES AND THE FORMATION OF THE JUVENILE THALLUS

Spores were removed from capsules after they had become brown, or at the time of the extrusion of the capsule through the involucre. The spores were placed on pieces of unglazed clay pottery in 0.1 per cent Knop's solution in petri dishes. The pottery had been superficially cleansed and then boiled in several changes of distilled water to remove any soluble matter. The water of condensation on the petri dish covers was periodically wiped off, and this loss regularly replenished with distilled water. Every two weeks the Knop's solution was discarded and fresh 0.1 per cent added. The cultures were kept out of direct sunlight, but exposed to bright diffuse skylight. No noticeable contamination of the cultures occurred except for several types of small *Diatoms* whose numbers never became great. A spore before it commences to germinate is shown in figure 102 a.

After a week to ten days the spores commence to swell thus becoming spherical. They are now more transparent and have become greenish. The swelling is a growth process and not a mere mechanical imbibition of water. In two weeks, a rhizoid is seen to break through the spore coat and elongate (fig. 102 b and e). Sometimes the formation of the first rhizoid is delayed until after a two or three celled sporeling has been formed (fig. 103 a, b, and e). With the increase in size of the spore, the spore coat, which at first may have been ruptured at one or more points, is now progressively digested; so that by the time the primary cell has divided, or even before (fig. 102 f), all that remains of the spore coat is the undigested papillae now irregularly scattered on the surface

of the sporeling, since the latter has increased considerably in volume (fig. 103). Sometimes most of the papillae are left grouped in one to several patches on the posterior cell or cells.

All spores germinate but spores sown together under identical conditions, did not all germinate at the same time nor develop with the same rapidity. This difference became more pronounced as the cultures became older. After a month, the more advanced thalli had formed a marginal growing region as in figure 105 while those most retarded were 4-6 celled thalli.

After  $2\frac{1}{2}$  weeks, the sporelings developing most rapidly had formed thalli of 2-5 cells (fig. 103). The numerous ellipsoidal chloroplasts are not drawn, but the exosporal papillae are indicated to show their scattering over the surface of the cells, and also to indicate the absence of a discarded spore coat. O'Hanlon's (45) figures for *Marchantia polymorpha* show no spore coat remaining on the sporeling, but she makes no remark upon the manner of its disposal. In *Fimbriaria* and *Targonia* however according to Campbell (10) and in *Reilla* according to Studhalter (53) and in others, the ruptured spore coats are left behind on the posterior cell of the sporeling.

The first division in the spore may or may not occur subsequent to an elongation of the spore cell. Rarely this elongation is pronounced enough to be considered as a "germ tube." The first divisions are very irregular. They usually follow one of two general lines of development. A filament of 3-5 cells may be formed, the anterior cell then dividing by a perpendicular anticline to initiate the broad thallus which is now developed in one plane by periclinal and perpendicular anticlines. In the second type of development the filamentous stage is eliminated altogether. The two celled stage is spherical and one or both of these cells may divide by walls perpendicular to the first, thus forming respectively two or four quadrants. Octants may now be formed, though usually one or more quadrant cells fail to divide thus resulting in a wide variation of forms. One to several of these octants or quadrants divide to form the broad thallus, the other cells rapidly mature. The posterior cell gives rise to a rhizoid, if this has not already occurred. Figures 104 a, b, and c show young thalli initiated from the filamentous type of germination. The original line of cell division here is rendered vague because the cells are distorted by turgor. Figures 104 d, e, and f show that the spore has developed directly into a thallus. Spores were germinated in two rooms: those exposed to longer periods of bright

skylight followed the latter mode of development, those exposed to shorter periods followed the former.

Occasionally longitudinal anticlines rendered the thallus two cells in thickness locally, though the thallus is often apparently or incompletely so due to the crowding and overlapping of these large turgid cells.

Figure 105 shows young thalli after further development of the anterior cells as they initiate a marginal growing region. The cells are becoming progressively smaller, but this decrease in size is overcompensated by the increase in number of cells, thus a progressively widening thallus is developing. Such a smooth growing margin as in figure 105 d and e is established in 5-6 weeks after sowing the spores. Two margins may be formed instead of one, thus resulting in branching. A transient apical cell is sometimes established early (fig. 104 g) but never persists, being soon replaced by the series of marginal growing cells in which no distinctly leading individual cell is discernible.

The thalli develop more rapidly where no actual film of water covers the plants. This was prevented by sowing spores over a cleansed, inverted flower pot kept in a covered glass jar at the bottom of which was half an inch of 0.1 per cent Knop's solution. The spores grow more rapidly at the top (base) of the pot where it was merely damp. No effort was made to control room temperature.

Thalli all grew in one direction in undisturbed cultures, indicating that light is the controlling factor in polarizing the young thallus: they grow perpendicular to the direction of incident light. Spores do not germinate in the dark.

With the formation of a meristematic margin, growth proceeds by each cell of this marginal row dividing by a wall parallel to the margin. The proximal series now divide by horizontal anticlines thus rendering the thallus two cells in thickness. These cells now mature. Most of the distal cells divide by vertical and longitudinal anticlines, thus producing nearly twice the number of marginal cells. Thus an individual row of cells near the margin as one passes from the posterior anteriorly appears to be incompletely dichotomous, though this effect is soon lost. Failure of one or more adjoining marginal cells to divide further initiates two branches. Rhizoids appear on the posterior ventral surface. They are thin-walled and smooth.

This investigation was carried out under the supervision of Dr. Duncan S. Johnson to whom I am indebted for his kindly interest and advice. I also wish to thank Dr. A. W. Evans for confirming the identification of the material studied.

## SUMMARY

1. *Dumortiera hirsuta* at Columbia, S. C., commences to form antheridia the last of August and liberates its spores the first of April. No strict seasonal development was observed in Jamaica, B. W. I.

2. The male or female receptacle originates as a multicellular meristematic process, the development of which is typical.

3. The development of antheridium and archegonium was traced, and this is found to be typically marchantiaceous.

4. No centrosome was seen in any mitotic divisions. The blepharoplast of the androcyte arises apparently independently in the cytoplasm. The elongation of the blepharoplast and elongation of the nucleus of the androcyte, and the formation of the mature sperm are typical for the *Hepaticae*.

5. Approach of the sperm nucleus to the egg nucleus and the fusion of these were observed. The fertilized egg underwent a brief period of rest before the formation of the sporogonium commenced.

6. The first two divisions of the embryo may form quadrants or a filament of three cells.

7. The development of the sporogenous tissue was traced and meiosis in the spore mother cells was observed. The first division in the latter was heterotypic, there being ten diads.

8. The maturation of the spores, elaters, and capsule is typically marchantiaceous.

9. The female receptacle elongates from 2-6 centimeters by an intercalary meristematic zone at the upper end of the stalk of the receptacle.

10. The seta of the sporogonium elongates solely by growth in length of these cells and not by cell division.

11. The capsule of the sporogonium opens into several irregularly split flaps.

12. The spores germinate in ten days in Knop's solution in strong diffuse light.

13. The germinating spore digests the exospore except for its papillae.

14. A flat juvenile thallus is formed that grows perpendicular to the direction of incident light by a marginal growing zone.

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## EXPLANATION OF PLATES

## PLATE 4

- Figs. 1, 2, 3, and 4. Longitudinal sections of young antheridia before first longitudinal anticlines are formed.  $\times 325$ .
- Fig. 5. Longitudinal section of antheridium showing first longitudinal anticlinal walls.  $\times 325$ .
- Fig. 6. Longitudinal section showing spermatogenous cells being cut out.  $\times 325$ .
- Fig. 7. Similar section showing spermatogenous tissue completely cut out.  $\times 325$ .
- Fig. 8. Longitudinal section of slightly later stage showing submergence of antheridium by more rapid growth of surrounding tissue.  $\times 325$ .
- Figs. 9, 10, 11, 12, and 13. Series of transverse sections through bodies of five antheridia showing steps in development. In figure 12 spermatogenous tissue is delimited.  $\times 325$ .
- Fig. 14. Median optical longitudinal section of a nearly mature living antheridium  $\times 85$ .
- Fig. 15. Longitudinal section of two young archegonia; involucre commencing to form at right.  $\times 325$ .
- Fig. 16. One apical cell of a female receptacle showing a two-celled archegonial rudiment at left.  $\times 325$ .
- Fig. 17. Similar section showing stalk of archegonial initial divided.  $\times 325$ .
- Fig. 18. Similar section with more advanced archegonium.  $\times 325$ .
- Figs. 19, 20, 21, 22. Longitudinal sections of further developmental stages of the archegonium.  $\times 325$ .
- Fig. 23. Cross-section of the neck of a mature archegonium.  $\times 325$ .
- Fig. 24. Same, before neck canal cells had disintegrated.  $\times 325$ .
- Fig. 25. Cross-section of the body of the archegonium of such a stage as figs. 21 or 22. One of the three original wall cells has divided.  $\times 325$ .
- Fig. 26. An archegonium as in figure 18 showing the three vertical walls in section.  $\times 325$ .

Figs. 27 and 28. Longitudinal sections of later developmental stages of the archegonium.  $\times 325$ .

Fig. 29. Longitudinal section of basal part of an abnormal archegonium with two eggs, two ventral canal cells, and a double series of neck canal cells.  $\times 325$ .

Fig. 30. Optical section of the tip of a living unopened archegonium.  $\times 325$ .

#### PLATE 5.

Fig. 31. Longitudinal section of a mature, opened, unfertilized archegonium.  $\times 325$ .

Fig. 32. Surface of tip of an opened archegonium.  $\times 325$ .

Fig. 33. Cross-section of the venter of an archegonium containing a fertilized egg.  $\times 325$ .

Fig. 34. Longitudinal section of venter showing egg and sperm soon after entrance.  $\times 325$ .

Figs. 35, 36, 37, and 38. Parts of similar sections showing approach of male nucleus and three stages of fusion with egg nucleus.  $\times 325$ .

Fig. 39. Longitudinal section of a two celled embryo.  $\times 325$ .

Fig. 40. Longitudinal section of a four celled embryo.  $\times 325$ .

Fig. 41. Longitudinal section of an eight celled embryo.  $\times 325$ .

Fig. 42. Not quite median longitudinal section of about a 20 celled embryo of the "filamentous" type.  $\times 325$ .

Fig. 43. Longitudinal section of an eight celled embryo of same type.  $\times 325$ .

Fig. 44. Transverse section near middle of a 30 celled embryo.  $\times 325$ .

Fig. 45. Longitudinal section of a 24-celled embryo of quadrant type.  $\times 325$ .

Fig. 46. Transverse section through the middle of a 52 celled embryo. Quadrant walls distinct.  $\times 325$ .

Fig. 47. Longitudinal section of an embryo of same type with capsule, stalk, and foot differentiating.  $\times 325$ .

Fig. 48. Longitudinal section of a later embryo showing sporogenous tissue distinctly.  $\times 325$ .

Fig. 49. Young androcyte showing diagonal cleavage between them.  $\times 1600$

Fig. 50. Androcyte after appearance of the blepharoplast.  $\times 1600$ .

Fig. 51. Blepharoplast near the nuclear wall.  $\times 1600$ .

Figs. 52, 53 and 54. Elongation of blepharoplast.  $\times 1600$ .

Figs. 55, 56 and 57. Elongation of nucleus. The blepharoplast has become associated with the nucleus.  $\times 1600$ .

Fig. 58. Distorted androcyte showing great elongation of blepharoplast.  $\times 1600$ .

Fig. 59. Further elongation of nucleus.  $\times 1600$ .

Figs. 60 and 61. Antherozoids from a dried smear. The anterior region is somewhat swollen.  $\times 1600$ .

#### PLATE 6.

Figs. 62, 63 and 64. Antherozoids from a dried smear. The anterior region is somewhat swollen.  $\times 1600$ .

Figs. 65, 66, 67, and 68. Young sporogenous tissue and elaters from various portions of the capsule.  $\times 325$ .

- Fig. 69. A portion of an elongated elater.  $\times 325$ .
- Figs. 70, 71, 72, and 73. First mitosis in a sporogenous cell that is to form a row of 8 spore mother cells.  $\times 800$ .
- Fig. 74. Several spore mother cell nuclei formed in such a sporogenous cell. The cross walls are not yet visible.  $\times 325$ .
- Fig. 75. Two of a row of spore mother cells increasing in size.  $\times 325$ .
- Figs. 76, 77, 78, 79, 80, and 81. Maturing of spore mother cells before meiosis.  $\times 325$ .
- Fig. 80. Terminal spore mother cell of a chain showing non-nucleate end of the parent sporogenous cell.  $\times 325$ .
- Fig. 82. Chain of spore mother cells in position as formed.  $\times 325$ .
- Figs. 83-97. Meiosis in spore mother cells.
- Fig. 83. The presynaptic spireme.  $\times 1600$ .
- Fig. 84. Synapsis.  $\times 1600$ .
- Fig. 85. Post-synaptic spireme.  $\times 1600$ .
- Fig. 86. Retreat of spireme to the nuclear wall.  $\times 1600$ .
- Fig. 87. Further retreat, paired chromomeres appearing.  $\times 1600$ .
- Fig. 88. Dyads formed.  $\times 1600$ .
- Fig. 89. Paired chromosomes on the heterotypic spindle.  $\times 1600$ .
- Fig. 90. Polar view of the heterotypic metaphasic plate.  $\times 1600$ .
- Fig. 91. Homeotypic prophase nucleus showing kinoplasm consisting of elliptical bodies after Gram's stain.  $\times 1600$ .
- Fig. 92. Metaphase of heterotypic division showing viscosity of chromosomes. Nine can be seen here.  $\times 1600$ .
- Fig. 93. Anaphase of heterotypic division. Ten chromosomes are present on both sides of the equator. One of the upper group lies under the others and is not drawn.  $\times 1600$ .
- Fig. 94. Formation of cell plate. Nuclei in prophase of homeotypic division.  $\times 1600$ .
- Fig. 95. Metaphasic plate of homeotypic division. Ten chromosomes appear in the figure.  $\times 1600$ .
- Fig. 96. Grouping of chromosomes in telophase of heterotypic division.  $\times 1600$ .
- Fig. 97. Elongation of daughter nuclei in preparation for homeotypic division.  $\times 1600$ .

#### PLATE 7

- Fig. 98. Metaphase of homeotypic division. Side and polar view of homeotypic spindles in same spore mother cell.  $\times 1600$ .
- Fig. 99. Three spores of a young tetrad.  $\times 1600$ .
- Fig. 100. Endosporium and exosporium formed, and episporium beginning to be formed.  $\times 1600$ .
- Fig. 101. Section through a mature spore.  $\times 1600$ .
- Fig. 102. a. A spore before enlargement has set in.  $\times 170$ . b-f. Stages in the rupture and dissolution of the spore coat of a germinating spore. First rhizoid appearing. These sporelings are two weeks old.  $\times 170$ .
- Fig. 103. 2-4 celled thalli  $2\frac{1}{2}$  weeks old. The undigested papillate thickenings of the exospore are represented as irregularly scattered dashes.  $\times 170$ .

Fig. 104 a-g. Initiation of the thallus. Thalli are four weeks old. g. Thallus showing ephemeral apical cell.  $\times 85$ .

Fig. 105. Thalli 5 and 6 weeks old showing the formation of the anterior marginal growing zone.  $\times 85$ .

PLATE 8

Unretouched photographs

Figs. 106 and 107. Median sagittal sections of the thallus showing young male receptacles.  $\times 47$ .

Fig. 108. Longitudinal section of a young female receptacle.  $\times 47$ .

Figs. 109, 110, and 111. Developmental stages of the sporophyte in longitudinal sections. The foot and seta are often wider than in fig. 111.  $\times 47$ .

Fig. 112. Annular thickenings in the cells of the capsular wall.  $\times 170$ .

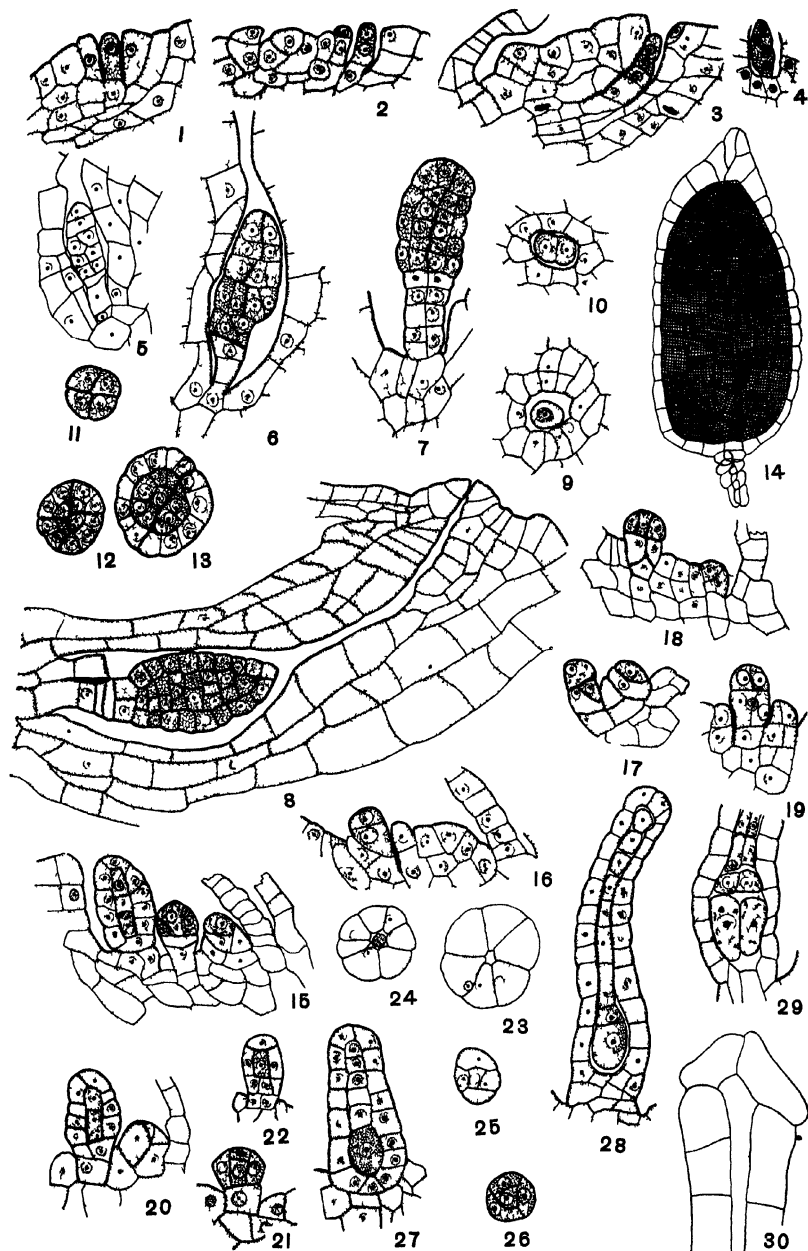
Fig. 113. Zone of contact of foot of sporogonium with the floor of the archegonium. Cells of seta are divided in preparation for elongation.  $\times 170$ .

Fig. 114. Spore tetrads and elaters.  $\times 170$ .

Fig. 115. Peg rhizoids from a furrow of the female receptacle showing copious mucilage unbleached from osmic blackening.  $\times 170$ .

Fig. 116. Similar section to figure 115 after bleaching and staining.  $\times 170$ .

Fig. 117. Three mucilaginous cells in a section of the involucre.  $\times 170$ .





# PLATE 5

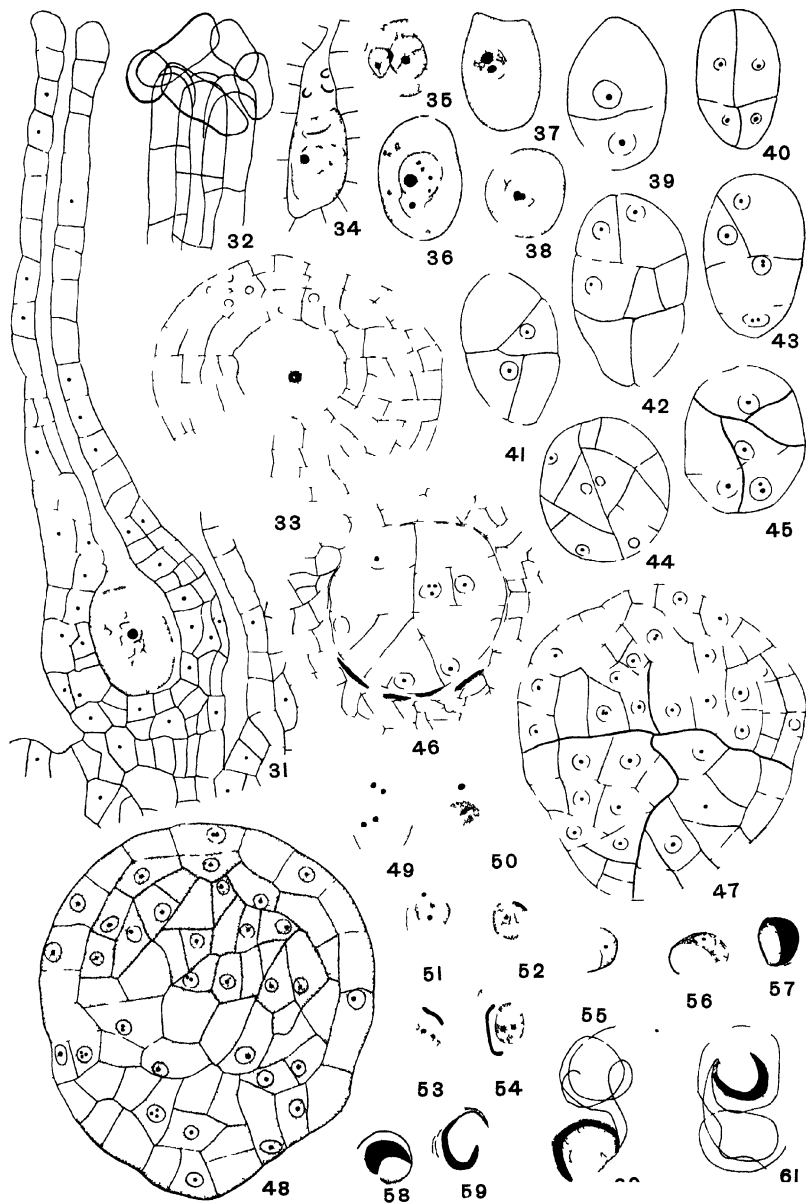






PLATE 6

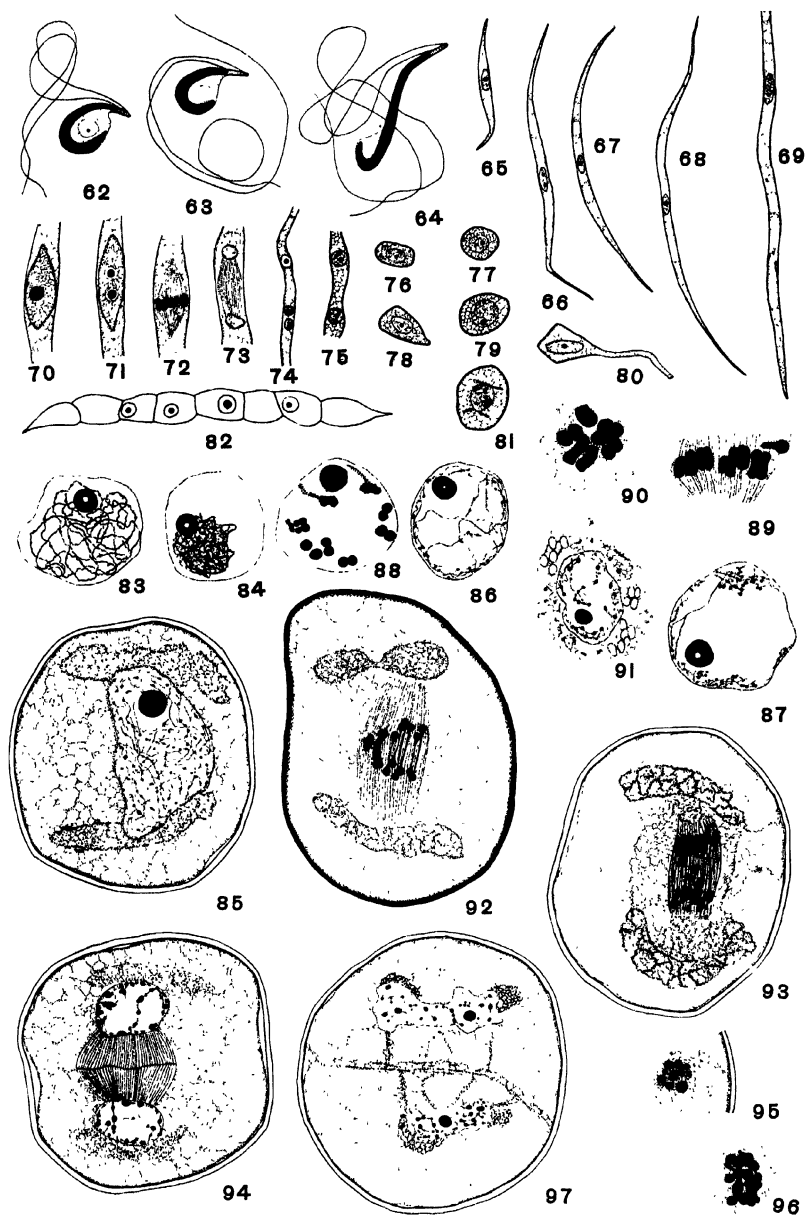
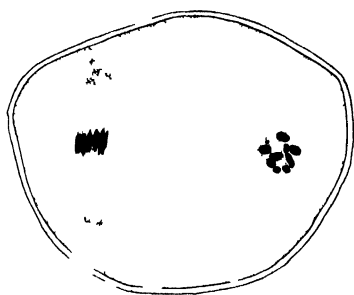
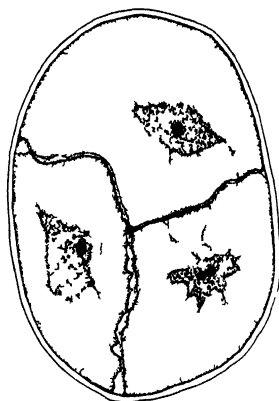




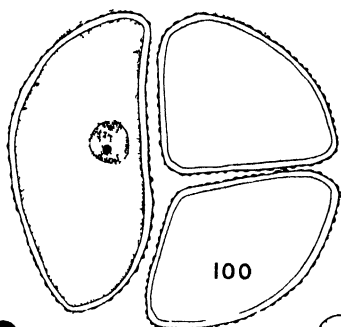
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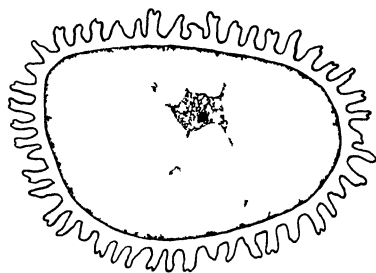
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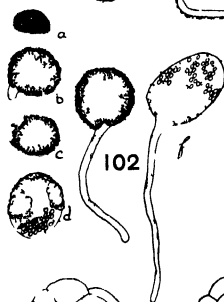
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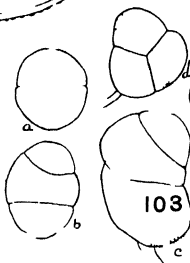
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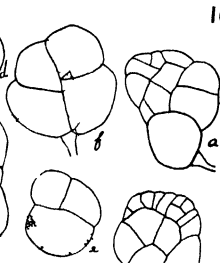
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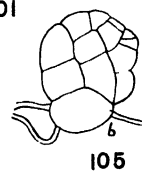
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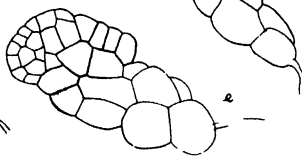
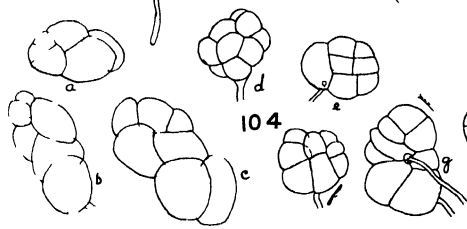
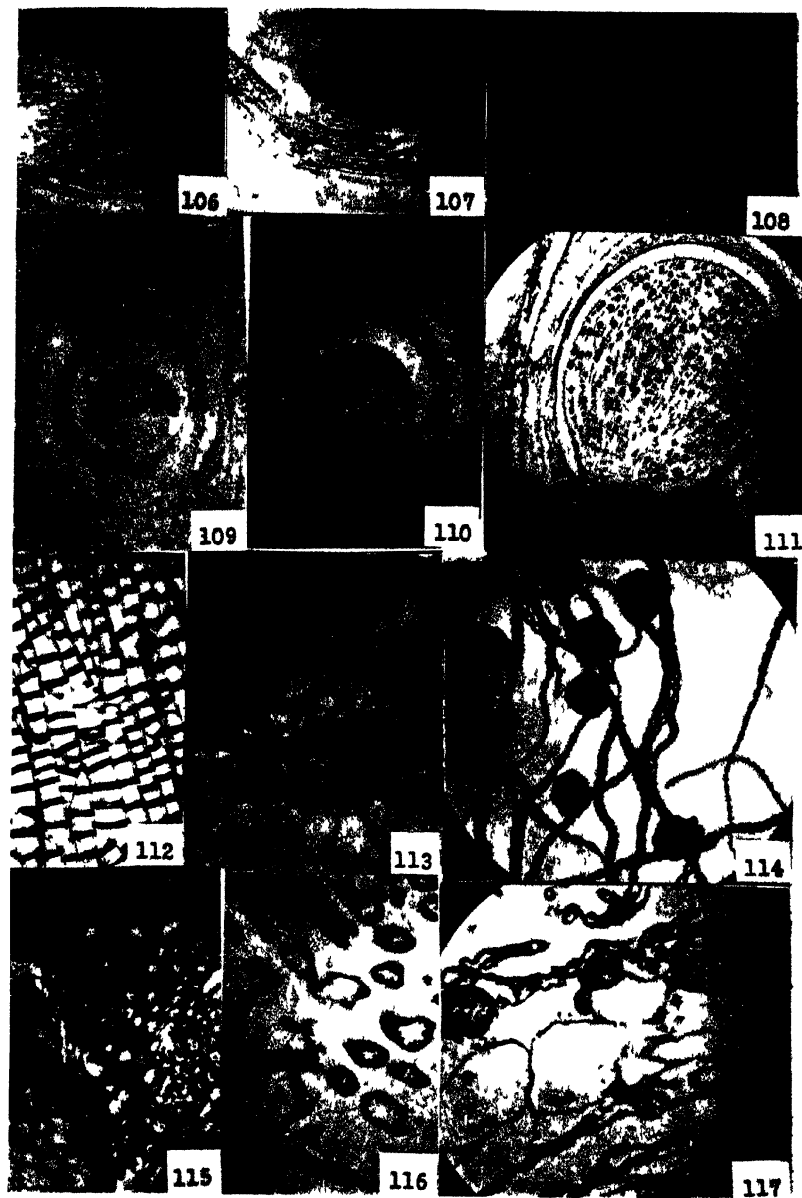




PLATE 8





PLEUROTUS TREMULUS FR. AND SECOTIUM CONICUM  
N. SP. FROM TENNESSEE

By L. R. HESLER

PLATE 9 AND 2 TEXT FIGURES

It appears that the gill-fungus *Pleurotus tremulus* Fr. is uncommon in the United States. Since ample material was collected near Knoxville, Tennessee, in the fall of 1932, it seems desirable to present descriptive notes prepared from fresh specimens.

*Pleurotus tremulus* Fr.

Plate 9 and fig. 1

**Pileus** 1-3.5 cm. broad, moist, subviscid in one collection only (No. 3687); fuscous to Chaetura drab (Ridgway) when fresh, cinereous when dry, hygrophanous; submembranaceous; conchate, dimidiate, or reniform, depressed behind and there white floccose-tomentose (under lens), other portions, in some specimens, appressed whitish fibrillose; margin undulate, often lobed and in some crenate-lobed, at first decurved then expanding somewhat, even or substrate. **Flesh** concolor (moist), pallid (dry), thin, homogeneous, without odor and taste. **Gills** adnate or decurrent, thick when young (suggesting *Cantharellus*) but thin and typically lamellate at maturity, many short, the shorter ones of about three ranks, subdistant, concolor (moist), paler (dry), sometimes inter-venose at the pileus, edge even, longer ones occasionally forked toward margin of pileus. **Stipe** 4-18 mm. long (commonly 4-8 mm.), up to 5 mm. in diameter, greyish, solid, dilated upwards, villose-strigose, lateral; stipe rarely lacking. **Spores** somewhat variable in shape, mostly pip-shaped and nearly spherical, or ovate-elliptical, a few pyriform; smooth, white in mass, usually apiculate; size somewhat variable, 6-8.5  $\mu$  x 3.5-7  $\mu$  (majority 7-7.5  $\mu$  x 5-5.5  $\mu$ ). **Basidia** clavate, about 22 x 7  $\mu$ ; sterigmata up to 5.5  $\mu$  long.

All collections were taken from a moss bed in pine woods near Knoxville, Knox County, Tennessee. (The moss species were determined by Mr. A. J. Sharp, of the University of Tennessee.) Specimens have been deposited in the University of Tennessee herbarium, as follows:



No. 3384, on moss (*Dicranum scoparium* [L.] Hedw. and *D. rugosum* [Hoffm.] Brid.) in pine woods, Nov. 15, 1932.

No. 3397, on moss (*Dicranum scoparium* [L.] Hedw. and *D. rugosum* [Hoffm.] Brid.) in pine woods, Nov. 20, 1932.

No. 3641, on *Dicranum scoparium*. Dec. 3, 1932.

No. 3687, on *Dicranum scoparium*. Dec. 11, 1932.

No. 3921, on *D. scoparium*, and on dead twigs among moss. Jan. 14, 1933.

Specimens collected in Tennessee vary slightly from the descriptions of Peck (1886) and Rea (1922). The substratum character of the margin of the pileus, exhibited by some collections, is mentioned neither by Peck nor by Rea. Although Peck states that the surface of the pileus is glabrous, Rea describes it as tomentose under a lens, which latter feature characterizes the Tennessee material. According to Rea, the spores are pip-shaped  $7-8 \times 3-4\mu$ ; Peck describes them as globose,  $7.5\mu$



Fig. 1. Spores of *Pleurotus tremulus* Fr. Specimen No. 3397. Drawing by Alice Caton.

broad. In the specimens here reported, the measurements come very well within the ranges recorded by Rea and by Peck. Neither author mentions an apiculus, commonly observed in Tennessee specimens. These differences, however, are slight and are regarded as insufficient to warrant the establishment of a new species, consequently it is referred to *P. tremulus* Fr. Moreover, on comparing my material with a specimen of *P. tremulus* Fr. collected in England, and now in the Atkinson Herbarium, Cornell University, close agreement in essential particulars was found. Inquiries of those in charge of several of the larger mycological collections in the United States indicate that specimens of *Pleurotus tremulus* are not in their herbaria.

I have not seen Peck's specimens, although Murrill (1916), who relegates the species to the doubtful realm, says that Peck's two specimens at Albany are very thin, gray, attached to moss, about  $3 \times 2$  cm., and that they resemble large forms of *Dictyolus muscigenus* Quél.



(ABOVE). *PLEUROTUS TREMULUS* FR. ON MOSS (*DICRANUM SCOPARIUM* AND  
*D. RUGOSUM*) SPECIMEN NO. 3397

Approximately natural size. Photograph by the author, Nov. 20, 1932.

(BELOW). *SECOTIUM CONICUM* N. SP. SPECIMEN NO. 3398  
Approximately natural size. Photograph by the author, Nov. 20, 1932



Murrill further comments that the lamellae are glued to the sheet and cannot be examined.

Nor have I examined Schweinitz's specimens, which are referred to by Murrill (1916), and which were collected in Pennsylvania. According to Professor Don M. Benedict (letter, April 20, 1933) these have not been found in the Schweinitz Herbarium at Philadelphia.

The Tennessee fungus has been compared with material labelled *Cantharellus retirugus* (Bull.) Fr. [= *Dictyolus retirugus* (Bull.) Qué.] from Dr. W. C. Coker, University of North Carolina, and with specimens from the New York Botanical Garden. Both of these collections exhibited gills of the type characteristic of the genus *Cantharellus*.

Illustrations of *Pleurotus tremulus* Fr. will be found in several European works. Schaeffer, who first described and figured the fungus from Bavaria (Fung. Bav. 4: Ind. 53; 3: pl. 224. 1774.), presents a rather poor illustration. Batsch's figures (Elen. Fung., pl. 24, fig. 123, a-c. 1786) are also poor; Sowerby (Eng. Fung., pl. 242, 1800) gives a fair representation, apparently about natural size. The species is also illustrated by Britzelmayer (Hymen. Sudbayan 1, pl. 42, fig. 292; pl. 65, fig. 384; and pl. 120, fig. 619. 1879?). Other figures, rather poorly done, will be found in Cooke (Illus. British Fung. 2: pl. 242. 1881-1883), Bernard (Champ. Rochelle, pl. 43, fig. 8. 1882.) and Juillard-Hartmann (Iconogr. Champ. Supérieurs 1: pl. 44, fig. 11. 1919.).

*Pleurotus tremulus* Fr. is somewhat closely related to *P. petaloides* Fr. The latter species, usually found on dead wood, has gills which are crowded, whitish or yellowish, and with fimbriate edges, and the pileus is a little larger than that of *P. tremulus*. From descriptions, it appears also to be close to *P. acerosus* Fr., but this species is silky white when dry, and exhibits globose spores.

Although *P. tremulus* has, since its discovery, been reported at occasional intervals in Europe, it has hitherto been found but twice in North America: at Poughkeepsie, New York, by W. R. Gerard (Peck, 1886), and in Pennsylvania, by Schweinitz (Murrill, 1916). Since it is rather inconspicuous, and likely to be overlooked, more rigid search may reveal its occurrence at other stations. It is usually reported as growing on moss gametophytes, on the ground, and on other fungi, such as *Auricularia caryophyllea* (vide Sowerby).

#### ***Secotium conicum* sp. nov.\***

\* The Latin description has been prepared by Dr. A. W. McWhorter, University of Tennessee, to whom I am indebted.

## Plate 9 and fig. 2

*Peridium* conicum ad convexo-conicum, basi truncata, alte excavata, pallido-cinereum ad ochraceo-cinereum, orbe fusciora et violaceo, 2-4.5 cm. altum, 2-4 cm. latum, albis sericeis fibrillis, viscidum. Caro 0.5-1.0 mm. crassa, pallida, gustatu subiucundo. Velum particulare album, arachnoide. Gleba fulva, labyrinthiformis, lamellaris, vel crasse cellularis. Stipes usque ad 5 cm. longus, 10-15 mm. per medium, fastigatus ad basim, pallidus, violaceus, subcavus ad cavum, subviscidus, subalbis sericeis fibrillis. Columella fastigatus ad apicem. Spori fulvi, ovato-ellipticales, crasse verrucoso-tuberculati,  $15 \times 11.25 \mu$  ( $13-20 \times 10-13.75 \mu$ ).

*Hab.* sub. *Pinu echinata* Mill., Knoxville, Tennessee, 300 meters, November 20, 1932, L. R. Hesler, 3398.

*Peridium* conical to convex-conical, sometimes slightly umbonate, base truncate, deeply excavated; pallid-cinereous to ochre-cinereous;

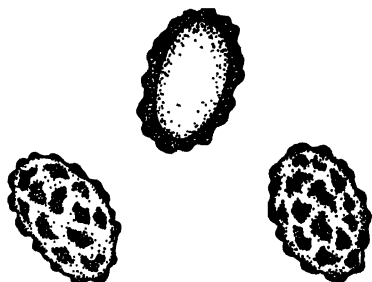


Fig. 2. Spores of *Secotium conicum* n. sp. Specimen No. 3398. Drawing by Alice Caton.

disk darker and when exposed tinged violaceous; 2-4.5 cm. high, 2-4 cm. broad; innately white silky fibrillose, viscid. Margin even or occasionally substrate. **Flesh** 0.5-1.0 mm. thick, pallid, taste slightly unpleasant. **Partial veil** white, arachnoid. **Gleba** brown (Prout's Brown, Ridgway) labyrinthiform, lamellar, or coarsely cellular. Dissepiments thin,  $\frac{1}{8}$  mm. or less in diameter. **Stipe** up to 5 cm. long, 10-15 mm. in diameter, tapering toward base, and there violaceous, elsewhere pallid, tinged violaceous, hollow-stuffed or hollow, subviscid, whitish silky fibrillose; columella narrowed at apex. **Spores** brown (Prout's Brown, Ridgway), ovate-elliptical, coarsely verrucose or tuberculate,  $15 \times 11.25 \mu$  ( $13-20 \times 10-13.75 \mu$ ). **Basidia** clavate, 41-52  $\times$  13-18  $\mu$ , 4 spored; sterigmata up to 2.8  $\mu$  long.

*Habitat.* On ground under oak (*Quercus* spp.) and pine (*Pinus echinata* Mill.). Solitary or gregarious.

*Type Locality.* New Hopewell neighborhood, Knox County, about ten miles southeast of Knoxville, Tennessee. Collections, at this station, were taken as follows:

No. 3377, on soil under pine, Nov. 9, 1932.

No. 3398, on soil under oak, Nov. 20, 1932.

No. 3575, on soil under oak, Dec. 3, 1932.

This species is distinct from *Secotium agaricoides* (Czern.) Hollos, which is rather generally distributed over the United States. Reference to the work of Cunningham (1924) indicated that the fungus here described might be *Secotium porphyreum* Cunn. The writer has received specimens of *S. porphyreum* from Cunningham and these have been compared with *S. conicum*. From this study, it is concluded that there are several points of difference, as follows:

*S. porphyreum*

1. Epispore coarsely verruculose.
2. Spores 12-17 x 8-11 u.
3. Peridium glabrous, depressed-globose.
4. Gleba color, Snuff Brown (R.).
5. Stipe tapering to apex, tinted yellow at base; columella slightly expanded at apex.

*S. conicum*

1. Epispore coarsely verrucose or tuberculate.
2. Spores 13-20 x 10-13.75 u.
3. Peridium fibrillose, conical to convex-conical.
4. Gleba color, Prout's Brown (R.).
5. Stipe tapering to base, tinted violaceous at base; columella narrowed at apex.

Although Cunningham (1924) makes no mention of a partial veil, his specimens, communicated to us, exhibit this structure.

THE UNIVERSITY OF TENNESSEE,

DEPARTMENT OF BOTANY,

KNOXVILLE, TENNESSEE.

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## BASIDIA OF SEPTOBASIDIUM (GLENOSPORA) CURTISII

By JOHN N. COUCH

### PLATES 10 AND 11

The genus *Glenospora* was established by Berkeley and Desmazières in 1849 on material collected by Curtis in South Carolina. The fungus has generally been considered to belong to the *Hyphomycetes*, in the family *Dermatiaceae*. Petch (1927) discovered that the fungus was associated with scale insects and was of the opinion that it should probably be referred to *Septobasidium*. Boedijn and Steinmann (1931) also found the fungus associated with scale insects on the branches of tea in Java. They considered the conidia or false-conidia, described by Berkeley and others, as probasidia. On the basis of these characters and the structure of the fungus they transferred it to *Septobasidium*. Neither Petch nor Boedijn and Steinmann were able to find any basidia and hence could not offer the final proof that *Glenospora* is a *Setopbasidium*.

The purpose of this paper is to present some notes on the distribution of "*Glenospora*," its structure and development including the development of the probasidia into basidia.

*Septobasidium Curtisii* (B. & D.) Boedijn & Steinmann, the species upon which the old genus *Glenospora* was based, is perhaps the most widely distributed and the commonest species of *Septobasidium* yet described. In collecting in the southeastern U. S. and along the Gulf Coast I have found lumbermen familiar with the black encrustations on the bark of trees, particularly black gum and ash. The fungus is so common that it would be difficult to find even a small stand of these trees not infested. It has been collected as far north as New Jersey, as far south as Guatemala, and as far west as Highlands, N. C. Boedijn and Steinmann (31) report the fungus from Java on tea. From the figures and description and from an examination of some material which the authors have sent me, their fungus appears to be correctly identified but since they did not get the basidia, the identity must remain uncertain.

A great variety of trees and shrubs are infested by this pest, as:

*Carpinus* sp; *Cornus amomum*, *C. florida*; *Crataegus Marshallii*; *Gleditschia triacanthos*; *Hicoria* sp; *Ilex opaca*, *I. verticillata*; *Juglans* sp; *Liquidambar styraciflua*; *Magnolia grandiflora*, *M. virginiana*; *Myrica cerifera*; *Nyssa aquatica*, *N. multiflora*, *N. sylvatica*; *Pyrus communis*, *P. japonica*; *Quercus phellos*, *Q. alba*, *Q. palustris*, *Q. rubra*, etc.; *Viburnum* sp.; *Thea* sp.; and numerous other unidentified plants.

The fungus forms resupinate, black or fuscous black, more or less circular patches usually between three and ten centimeters in diameter on the living bark. Sometimes, however, the growth may cover an area as much as thirty centimeters wide. It is entirely superficial, not penetrating at all into the tissue of the bark, a fact easily demonstrable by sections. The surface characters vary considerably. Sometimes the surface is more or less dotted with upright spines which may arise singly or in clusters. The spines may be so few and small as to be barely visible without a lens or may be abundant and conspicuous (up to 1 mm. tall). The specimens with a spiny surface usually occur on *Quercus* or *Magnolia* and rarely on *Nyssa*. More commonly, however, the spines are absent and the surface is roughened by irregular cracks and holes or tufts of loosely woven hyphae or tangles of fascicles of hyphae as in the forms on *Fraxinus* and *Nyssa*. Sometimes the surface may be nearly smooth. The subiculum in the marginal region during the growing season is purplish slate colored with the outermost edge whitish. This marginal region is characterized during this season by the presence of numerous, irregularly shaped, minute enclosures which are pale brownish on top and are usually arranged in one or more irregular, circular rows. Under a strongly magnifying hand lens or a dissecting binocular, this marginal region looks like an airplane view of an Indian village. Some of the enclosures are at first V-shaped or U-shaped or semi-circular or like an open umbrella; but as they increase in size their margins anastomose so that they become irregularly shaped. Often the tops of these structures are bordered by radiating fascicles of hyphae, thus giving the appearance of a series of stars. Petch (1927) called attention to these peculiar structures. As a rule the roofs of these enclosures grow out horizontally, anastomosing with each other and with the older part of the fungus and in this way the top layer is formed. Sometimes, however, as in the form on *Magnolia* the enclosures are so scattered that their roofs never fuse with each other and thus the fungus consists of a subiculum and the scattered enclosures.

The more or less indefinite hymenial region may be formed over the top layer or if there is no top layer, as in the form on *Quercus* or *Magnolia*,



the spore bearing organs are formed on the upright spines and the subiculum. The probasidia are formed during the fall and early winter and germinate in the spring and early summer. They begin to germinate about the latter part of March with the advent of the warm spring rains. Spore formation reaches its peak about the first half of May. From this time on there is a gradual decrease in the number of spores formed until July 1 when practically all of the probasidia have germinated. These records apply only to Chapel Hill. In a large collection of material sent me November 3, 1932, by Dr. B. B. Higgins from near Vaughan, Georgia, I found large numbers of mature probasidia which could be made to germinate into basidia. The basidia are long cylindrical, and four celled. Each cell gives rise to a spore which, instead of germinating into a hyphal thread becomes divided, as a rule, into four cells and produces numerous yeast-like bud cells.

As mentioned above both Petch (1927) and Boedijn and Steinmann (1930) have noticed the scale insects beneath the stroma of *S. Curtisii*. As in the case of *S. retiforme* (B. & C.), Pat., the fungus and scale insects live together apparently in a mutually symbiotic relation (Couch 1931). Since a good many important points in the relationship between the fungus and insects remain unsolved, a detailed report on this subject will be given in a later paper.

The fungus-insect combination causes considerable damage to certain trees and shrubs, particularly species of *Fraxinus* and *Nyssa*. The commonest type of injury shows itself as a cracking of the bark with a marked hypertrophy of the underlying tissue and sometimes the injury may show itself in the form of witches brooms.

*Septobasidium Curtisii* (B. and D.) Boedijn and Steinmann

*Glenospora Curtisii* Berkeley and Desmazières.

Fungus resupinate, thin, growing in effused patches which often cover an area equal to 30 or 40 square centimeters. Surface nearly smooth in some specimens but more often dotted with minute areolations, considerably cracked in old specimens (in some specimens, especially those on oak, the surface is roughened by upright pillars of hyphae); usually black with a purplish tint (about plumbeous black of Ridgway) but often fuscous. Margin variable, sometimes thick, terminating abruptly and then concolorous with the main surface, usually thin during the growing season and of a lighter color than the main surface, whitish with a purplish sheen toward the outer edge, and sometimes indeterminate. Marginal region dotted with numerous patches of elevated hyphae which

sometimes are star-shaped, umbrella-shaped, tent-shaped or shaped like an Eskimo hut. In section about  $200-400\mu$  thick, sometimes compact throughout but more often composed of three more or less distinct regions: (1) the subiculum which grows horizontally over the bark, very thin, hyphae of subiculum  $4-5\mu$  thick in marginal region with much anastomosing between threads; (2) the middle region which arises from the subiculum sometimes as short thick pillars, the threads of which quickly branch out to form the top layer; (3) top layer  $60-300\mu$  thick, the thickness depending upon the age, threads of top layer and pillars about  $4\mu$  thick; in young specimens composed of only one hymenial region while in old specimens the top layer may be stratified by the successive formation of several hymenial layers. Hymenium composed of probasidia and basidia. Probasidia formed during the fall and early winter (young, small, thin walled Oct. 10, mature Dec. 10), germinating into the basidia during the spring and early summer; mature probasidia  $10.8-14.2\mu$  thick (most about  $12\mu$  thick), spherical, wall about  $1.5\mu$  thick, often with numerous minute furrows on the inner side which give the wall, in a section view, the appearance of being pitted; basidia  $6.3-7.6\mu$  thick by  $62-70\mu$  long counting the stalk, straight, thickest in the middle, often breaking off from the probasidium and its stalk; stalk  $10-20\mu$  long; basidium becoming divided by three transverse walls into four cells, each cell sprouting a short pyramidal shaped sterigma, which is very peculiar in that, after the formation of the basidiospore, the sterigma remains full of protoplasm and then buds several very minute sporidia; basidiospores hyaline, usually bent elliptic, becoming once or thrice septate,  $3-4.2 \times 13-21\mu$  (coll. No. 8315, Chapel Hill). Conidia formed over the lower surface of the fungus, globose, borne in chains.

Associated with *Chionaspis sylvatica* Sanders, *C. gleditsiae* Sanders, *Chrysomphalus obscurus* Comst., and *Aspidiotus* sp. on a large variety of trees and shrubs.\*

The present species because of similar habitat and color may be confused with *S. Patouillardii* Burt. Burt's material of *S. Patouillardii* was collected on living branches of *Fraxinus*, *Liquidambar*, and *Nyssa*, which hosts I have found to be very common ones for the present species. Burt (1916) describes his plants as being "aniline-black at first, becoming fuscous in the herbarium," colors which agree with the present species. The two plants may be separated by gross and micro-

\*The scale insects have been identified by Dr. Harold Morrison, Bureau of Entomology, U. S. D. A., Washington, D. C.

scopic characters. *Septobasidium Patouillardii* Burt always shows a distinct differentiation into three layers, the top layer being supported by pillars. In the present species the layered condition is often quite indistinct and the plant is never distinctly three layered throughout. In probasidial and basidial characters the two plants are quite distinct. In *S. Patouillardii* Burt the probasidia are small and elongated in shape; in the present species they are large, distinct, thick-walled, and globose. In the former the probasidial cell elongates to form a two celled basidium, the probasidial cell therefore does not persist after the formation of the basidium; while in the present species the four celled basidium sprouts from the probasidial cell, the latter persisting for a long time as an empty cell after the basidium has matured.

This species can be recognized by the deep fuscous or plumbeous black color and by the very thin structure.

Through the kindness of Dr. John A. Stevenson, I have been able to examine the following specimens of *S. (Glenospora) Curtisii* (B. & D.) B. & S. from the Herbarium of the United States Department of Agriculture.

- On *Magnolia*, Louisiana (?). Langlois coll., Nov. 4, 1888. Many empty probasidia, no basidia.
- On *Nyssa*, Darien, Ga. Ravenel coll., No. 333. Probasidia very abundant, 10.5-13 $\mu$  thick, often slightly rough, and with a brownish purple tint under the microscope. No basidia seen.
- On *Nyssa*, Hyattsville, Md. F. L. Scribner coll. Many probasidia 10-13.4 $\mu$  thick, as in Ravenel material.
- On *Nyssa*, living branches, near Burgaw, N. C. W. W. Diehl coll. and det., April 25, 1930. Like Chapel Hill, N. C., material.
- On *Nyssa*, living branches, Auburn, Lee Co., Ala. F. S. Earle and C. F. Baker colls. (No. 2240), Jan. 16, 1897. Typical of Chapel Hill material.
- Oh *Nyssa multiflora* Walt., living branches, Newfield, N. J. J. B. Ellis coll., winter of 1874. (De Thümen, Mycotheca Universalis, No. 292) Probasidia abundant, 10-14 $\mu$  thick, many 12.6 $\mu$  thick; no basidia. Associated with *Chionaspis* sp., many of which are not parasitized and are giving birth to young. Like Chapel Hill material on ash and *Nyssa*.
- On *Nyssa sylvatica*, living branches, Auburn, Ala. L. E. Miles coll., Dec. 5, 1922. Typical. Associated with *Chionaspis sylvatica* (?).
- On *Nyssa sylvatica*, living limbs, Clarendon, Va. J. R. Weir and W. W. Diehl colls., April 16, 1923. Det. by Diehl. Brownish. (Two other collections from Va.)
- On *Nyssa sylvatica*, living limbs, Monroe, La. E. L. Dennison coll., Jan. 30, 1928. Det. by J. A. Stevenson.
- On *Carpinus*, living twigs, Pointe a la Hache, La. Langlois coll., April 20, 1888. Many probasidia; no basidia seen.

- On *Carpinus*, Darien, Ga. Ravenel coll. Resupinate with no upright pillars.
- On *Gleditsia triacanthos*, living branches, Louisiana. Langlois coll. (No. 179), Jan. 5 and 6, 1886. Material abundant. Probasidia spherical, 10-13 $\mu$  thick, some minutely spiny. No basidia seen.
- On *Juglans*, living branches, West Raleigh, N. C. F. L. Stevens coll., Jan. 8, 1908. Det. by A. Ames. Like Chapel Hill material.
- On *Pyrus communis*, living limbs, Cairo, Ga. Ogara and Worth colls., Feb. 2, 1903.
- On *Viburnum*, living twigs, Fort Valley, Ga. J. C. Dunegan coll., Jan. 19, 1922. From herbarium of L. O. Overholts (No. 8114). Also another collection March 18, 1922.
- On bark of trees, Yadkin River power house, N. C. P. O. Schallert coll., July 4, 1925.
- On deciduous tree, Florida. Calkins coll., winter of 1886.
- On angiosperm tree, Guatemala. W. A. Kellerman coll., Dec. 28, 1906. Det. by W. W. Diehl as *Glenospora*. Pillars very abundant, about one millimeter tall. Hymenium forming a smooth surface over tops of pillars. Probasidia very abundant, spherical, 8.4-12.6 $\mu$ , most about 9 $\mu$  thick, color and wall characters as in Ravenel plants. Basidia not seen. Before positive determination can be made, basidia should be studied.

## SUMMARY

*Septobasidium Curtisii* (B. & D.) Boedijn and Steinmann is perhaps the most widely distributed and the commonest species of *Septobasidium* yet described. It has been reported on a large number of trees and shrubs, sometimes causing considerable damage.

The basidia are described for the first time. The probasidial cell (conidium of Berkeley and others) germinates and empties its contents into a cylindrical structure, the basidium, which becomes thrice septate and thus four celled. Each cell gives rise to a sterigma on the end of which a basidiospore is borne, after which the sterigma which remains full of protoplasm may give rise to several very minute sporidia. The fungus is perennial and in this locality the probasidia are formed in the winter and germinate in the spring and early summer. These observations afford final proof that Petch and Boedijn and Steinmann were right in placing *Glenospora Curtisii* Berkeley and Desmazières in the genus *Septobasidium*.

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## EXPLANATION OF PLATES

## PLATE 10

*Septobasidium Curtisii*. Two pieces on left on ash (*Fraxinus americanus*). Collected and photographed July 18, 1928, by A. B. Couch (No. 8343, nat. size. The half-moon shape of the growth is due to the fact that one-half of it was cut away the preceding year). Note whitish margin—new growth zone for season 1928. Note also upright tufts of hyphae forming fungal houses in marginal zone. Two pieces on right on black gum (*Nyssa sylvatica*), collected and photographed by the author Feb. 1933 (No. 9349, slightly reduced). Note large cracks in bark. Margin of fungus concolorous with other parts during dormant season.

## PLATE 11

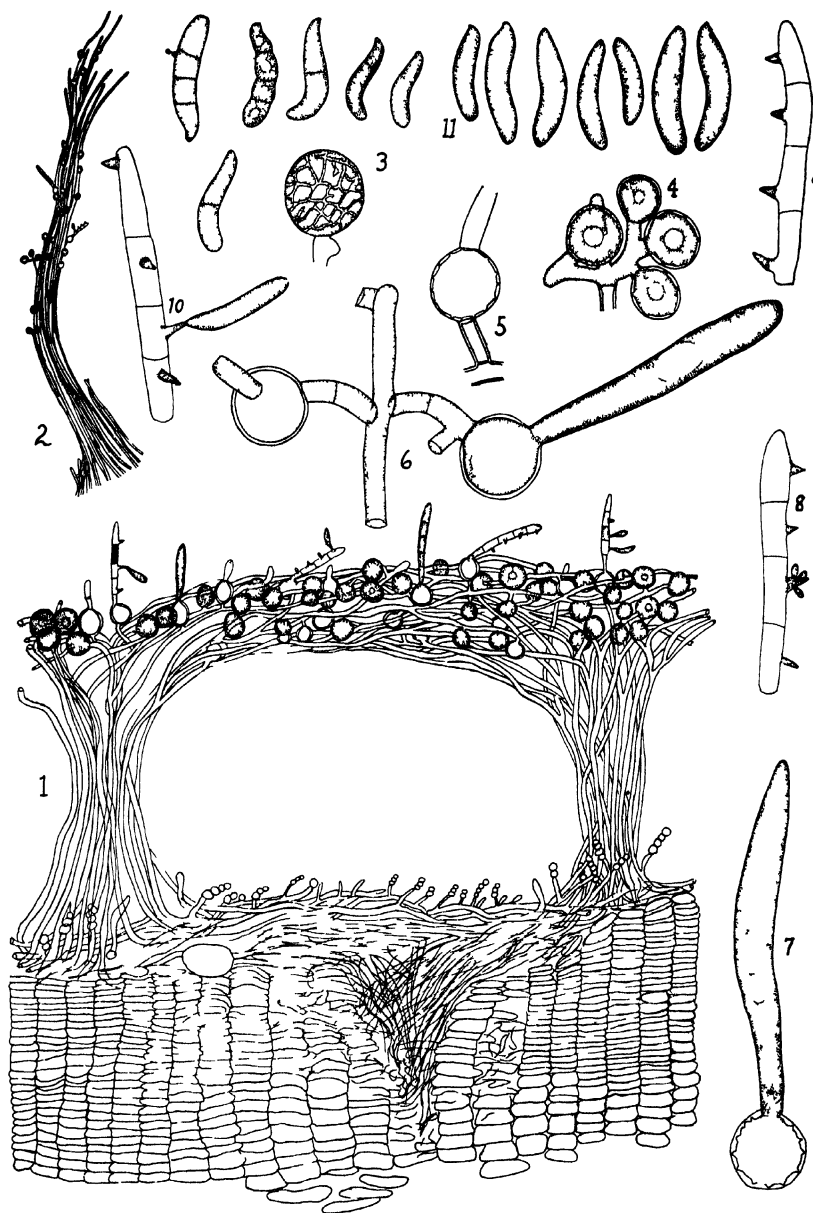
- Fig. 1. Section through fungus showing pillars, hymenium with probasidia, basidia, spores, and conidia.  $\times 234$ .
- Fig. 2. An upright spine on which are several probasidia and basidia.  $\times 100$ .
- Figs. 3, 4. Probasidia, the latter showing the shiny globules mistaken for nuclei by Berkeley. (Ellis, No. 292).  $\times 835$ .
- Fig. 5. Empty probasidial cells showing stalk left after basidium has fallen off.  $\times 835$ .
- Figs. 6-10. Basidia in various stages. Figs. 6 and 7, young basidia. Fig. 8 shows minute bud cells on sterigma after spores have been formed and fallen off.  $\times 835$ .
- Fig. 11. Spores.  $\times 835$ .

PLATE 10





PLATE 11







## SEXUALITY OF ALLOMYCES ARBUSCULA BUTLER\*

By WINSLOW R. HATCH

### PLATE 12

In 1929 Kniep (3) observed a unique type of sexuality in a new species of *Allomyces* (*A. javanicus*) which he discovered in Java. He recognized the close similarity between his species and *A. arbuscula*, but since Butler (2), Barrett (1), and more recent investigators had failed to observe sexuality in *A. arbuscula*, Kniep felt justified in giving his plant a new name. He still persisted in the opinion, however, that sexuality would probably be ultimately found in *A. arbuscula*. He pointed out that gametangial development depends most certainly on a very definite set of external conditions, suggesting that the failure of other workers to observe sexuality in *A. arbuscula* might be due to the absence of conditions favoring gametangial formation.

Since Kniep's remarkable discovery no one has been able heretofore to confirm his results. In the present paper his observations are confirmed in nearly all details, though perhaps on a different species of *Allomyces*.

The material on which the following observations were made was collected from a branch near Raleigh, N. C., July 7, 1931, and has been kept in culture in the Botanical Laboratory at the University of North Carolina since that time.

From these cultures material was selected and brought to this laboratory by Dr. John N. Couch, who, at the time, was engaged at this University in the supervision of a course of study on the Phycomycetes. As laboratory material, these cultures were subjected to careful examination but no signs of sexuality were observed. The material arrived here April 7th. After having been studied by the class, it was transferred once on the 17th, but the cultures were left in the laboratory and were given no further care until April 27th, at which time distilled water was added to them. From April 27th to May 20th they again fell into neglect. Only once during that period (some time between May 5th and 13th) was the old water drained off and fresh water added.

\* Botanical contribution from the Johns Hopkins University No. 122.

It was at this time that the gametangia were first noticed. Their presence is not a thing that could be easily overlooked and a casual microscopic examination was enough to attract attention to them. The apparent "sporangia," characteristically associated in pairs, were strikingly colored, one a distinct salmon pink and the other an ashen gray. Having observed these structures, I was prompted to sketch them but even then their significance was not fully realized. And so, in the rush of work, the cultures were forgotten again until May 20th, at which time this work was begun.

By the 20th of May only two Petri dish cultures had survived and these showed no signs of the striking gametangia that had been seen earlier. In the interim of possibly a fortnight, all had discharged and only their colorless walls remained to testify to their existence. Hemp seeds were added to these old cultures and new cultures thus started. Gametangia appeared on the new cultures four days later.

The gametangia appear at the tips of the hyphae and most frequently occur in pairs consisting of one male and one female gametangium. They may, however, become associated into chains of as many as six or more. It is characteristic in this species for female gametangium to be terminal. It is always subtended by the male and, in the event that chains are built up, a male alternates with a female throughout their length. Occasional exceptions to this regular order do occur, however, as when two males form beneath one female.

The fact that the male and female gametangia are of different colors has already been mentioned. There is also a constant difference in shape. The female gametangium is always the more oval structure of the two and sometimes it is quite spherical. The male is sometimes rather oval but more often its diameter does not greatly exceed that of the hypha from which it was cut off.

In an actively growing hypha the terminal portion has a dense protoplasmic content. From such a filament the gametangia are cut off by two cross walls that come in just back of the tip. Following upon the appearance of these walls both terminal cells show a distinct bulging of their lateral walls. Particularly is this true of the terminal cell. As the gametangia increase in size the subterminal cell becomes colored, taking on first a yellow tint and gradually changing as development proceeds until the final salmon pink color (rusty under low power) is attained. This color is much more distinct with daylight than with artificial light.

Next one to several papillae appear. They gradually grow more

and more pronounced. The color distinctions are generally very definite at this time, but occasionally papillae appear before marked color changes have been initiated. In still other instances no absolute color distinctions arise, but this is rare and limited to abnormal thalli.

The gamete origins seem to be indicated by a large number of peripheral vacuoles surrounded by granules. In the center of each vacuole is a hyaline mass of material, perhaps the nucleus. In one female gametangium between 50 and 60 of these vacuoles were counted. The vacuoles in the female are about twice the size of those in the male gametangium. The central region of each gametangium is occupied by faintly granular cytoplasm. During this stage a distinct rocking and jerking motion is visible on the part of the male gamete origins. The female origins remain practically motionless or at most show very faint movements. It must be clearly understood that these vacuoles surrounded by the granules merely indicate the position of gamete origins and are not the origins themselves. The gametes are two to three times as large as these vacuoles.

Just as the vacuoles surrounded by the granules have reached a fine degree of distinctness they go into a disappearance stage. Within a minute or so all outlines are lost and the gametangia appear uniformly granular. This is an abrupt change. It is, however, of only about 2-10 minutes duration. At the end of that time the outlines of the gametes become visible. As they appear it is obvious that they are much larger than the vacuoles which indicated their position. The gametes are not, however, nearly so distinct as one might expect, nor do they appear to become distinct until discharge has partially emptied the gametangium.

Normally, discharge follows very soon after the reappearance of the origins in their mature form. But in the interval before discharge there is an incipient movement within the gametangia and among the gametes. Sometimes the two gametangia of a pair may discharge almost simultaneously; at other times either the male or female may discharge first. After the bursting of the papillae of emergence by the first gamete there is a rather steady egress of gametes for a time but as the gametangia are gradually emptied the intervals between the escape of the gametes at any one pore become longer. Thus while the majority may have escaped in the first 10 minutes, a few (1-3 or more) will still be found in the gametangium 20-30 minutes later.

The male gametes appear one at a time, sometimes close behind each other but nevertheless always distinct. In making their escape they have first to push through a small pore, the narrowness of which con-

stricts them in the process. But even when outside, the gamete is not always free, since very frequently it has trouble in disengaging its long cilium which it drags behind while pushing out of the gametangium. Indeed, 4 hapless gametes have been seen to tug for 14 minutes before they could free their cilia. The male gametes are more active than the females. Their activity starts in the gametangium itself just as soon as partial discharge has made any considerable movement possible, and they move around jerkily in these close quarters.

The female gametes are slow to escape. Upon discharge they are at first perfectly quiet. Then they begin to swing slowly on their axes through an arc of not more than  $45^{\circ}$ . This movement ultimately gives way to a rapid vibratory motion and finally the gametes move off, each propelled by its single, posteriorly attached cilium. Sometimes the gametes emerge before they have become completely differentiated; in such event they attain their individuality out in the water.

As might be expected, the general vigor of the gametes differs with different gametangia and particularly with different cultures. Hence there is no absolute conformity in the duration of their swarming period or in their activity during that period. The duration of the swarming period for unconjugated females was found to be short, however. In one extreme case the female gametes issued from the gametangium very, very slowly; they were more than ordinarily delayed in individualizing themselves from the mass in which they escaped. Once free they showed the weakest sort of ciliary movement and came to rest almost immediately without conjugating and without going out of the field of the microscope at 400 magnification. In most cultures there is a longer active period and the females show vigorous ciliary motion. As regards the males, in the best instance that could be held under observation they came to rest in 22 minutes, without going out of the field of the microscope at 80 magnification. Even the lack of female gametes in their immediate vicinity did not stimulate them to more prolonged activity. But even after the gametes come to rest motion does not entirely cease. The males show a weak ciliary movement for at least 10 minutes and the females much longer, especially in the event that they conjugate.

The gametes differ in size, color, granulation and, less markedly, in motion. The female is 2-3 times as large as the male, and is of a gray color, which has a greenish gleam about it. The male, on the other hand, can often be distinguished by its brassy color. The female is finely and diffusely granular while the male is more coarsely so, but both show a clear hyaline area on occasion—sometimes anteriorly and sometimes posteriorly located.

Observations on the motion of the gametes are not in complete agreement, due probably to inherent differences in vigor of the products of different gametangia and also no doubt due to the possibilities for motion offered. When in close confinement, the motion is different than when free-swimming. In a pair of gametangia hemmed in on all sides by hyphae and detritus the motion of the male gamete may be described as a darting movement. The gametes darted forward for a short distance then vibrated in approximately the same position. This procedure was then repeated. For the sharp darting movement the cilium, which had been allowed to swing forward in a loose loop, was snapped back. Then, held hard back, it was whipped around in small circles, thus imparting a vibratory or "wriggling" motion to the gamete itself. The motion of the female was like that of the male but less rapid.

When the gamete is in the open, however, its motion is easier and the gamete glides along with no interruption to its forward progress. It also apparently turns on its axis as it moves ahead. This was especially evident in a free-swimming fusion product, on which a slight bulge marked the position of the male. This irregularity of contour helped to establish the rotary movement of the sexual swarmer on its own axis.

The gametes also have the power of amoeboid movement. This is particularly marked in the female and is not dependent on fusion. Certain unfused female gametes which came to rest almost immediately were seen to adopt very striking amoeboid movement. This was accomplished by the extension of a hyaline pseudopod-like process into which the main bulk of the gamete (i.e., the granular portion) streamed. This phenomenon was initiated about 20 minutes after coming to rest. The ciliary movement, always weak, practically ceased when the amoeboid movement set in. This movement, however, is most marked in the case of conjugating gametes and will be described later.

It has already been mentioned that very frequently the paired or chained gametangia discharged their contents simultaneously, in which case the fusion of the gametes is made easy. It has also been noted that the female gamete is slow, after its discharge, to free itself and to start its individual, free-swimming existence. This habit makes fertilization still easier for the males since it is they, usually, that seek out the female gametes. The following observations will describe the behavior of the gametes during fusion:

9:49 a.m. The last gamete in the female gametangium started pushing through the exit pore.

9:50 a.m. Before it could free itself a male moved up and apparently became attached by its anterior end (fig. 12).

10:01 a.m. The two became separated but only momentarily, the male again applying itself to the female directly, this time with a vigorous lashing of its cilium, as if it were really trying to force an entrance. This in turn induced a spasm of violent bucking movements in the female, then both became quiet and so closely appressed that it was thought that fusion had been completed (fig. 13). As a matter of fact, it had just begun.

10:15 a.m. The female began to blister and bubble over its whole surface (fig. 14). This was the start of the amoeboid movements that were going to continue for 10 minutes. Ciliary motion, however, was not abandoned. The associated cells now became much elongated (figs. 15 and 16). In this form they thrashed and whipped about, accompanying these motions with amoeboid movements that, on occasions, drew out the fusion cell into very odd shapes. One shape often seen at this time was that of a bent rod (fig. 16). This cell had clear hyaline caps at both ends and consisted of 2 distinct granular portions connected by a narrow isthmus. A distinction could sometimes be seen between the granulation of these two areas, one being coarser than the other. The two attenuated ends moved as if they were both ciliated, but only one cilium could be seen, so that it may well be that the beating movements were merely transferred to the opposite end without originating there. Another shape assumed was that of figure 17. At this stage only one cilium could be seen and that issuing from the lighter end—the one with the many amoeboid processes. In all these violent movements the fusion cells moved about over the parent gametangium.

10:25 a.m. The zygote became noticeably quiet and started sliding down over the surface of the gametangium towards the hypha (fig. 18).

10:30 a.m. It rounded up and went into encystment, 40 minutes after fusion started (fig. 19).

Another pair of gametes, the female from this same gametangium, was also followed through this long-drawn-out process of fusion. The process took a full hour in this second case.

At other times, when one gametangium precedes the other in the discharge of gametes, the fusion would seem to be almost impossible of accomplishment. Particularly would this be true if a pair of gametangia were isolated. I tried just such an experiment, isolating a single couplet and waiting upon dehiscence. The male preceded the female in dehiscence by 32 minutes. Under these circumstances the accomplishment of conjugation was most interesting.

9:23 a.m. The discharge of the male gametangium began.

9:42 a.m. Only 3 gametes were left in the male gametangium.

9:45 a.m. By this time the male gametes which had all been seen to move off in the same direction and which had dropped out of the focus of the gametangia were found to have dropped to the bottom and to have sought the light. There were 28 male gametes in the field of the microscope at 80 magnification. They were already coming to rest.

9:50 a.m. All the male gametes were sluggish but still moving.

9:55 a.m. The female gametangium discharged a single gamete.

10:00 a.m. General discharge occurred.

10:10 a.m. The female gametes were found to have followed the path of the males. Having sought out the males, all but 1 or 2 of the late arrivals were already fusing with them. A characteristic uneasy motion among the different fusion cells next became apparent as they clumped with each other, freed themselves, then clumped again. Here too there were manifested those characteristic amoeboid movements and distortions of shape described in the first case of gamete fusion. Ultimately, as a result of the extension of pseudopod-like processes about the male, that gamete is gradually absorbed by the female.

10:45 a.m. The single cilium was still in evidence though showing very slow movements.

11:00 a.m. Fusion was not yet completed, the males much reduced in size but still distinct (figs. 7-11).

Fusions have also been observed in free-swimming gametes. This statement might better be qualified by explaining that fusion cells have been seen in the free-swimming condition. Although the male was quite prominent it did not necessarily hamper the movements of the female whose motion continued to be free and easy. The germination of the zygote follows very promptly, possibly within a half hour, and certainly within an hour, as described by Kniep.

I wish to thank Prof. John N. Couch for suggesting the problem. As soon as notified of my observations Prof. Couch examined material in Chapel Hill and has confirmed in general the observations described in this paper. Prof. W. C. Coker also observed the fusion of the gametes.

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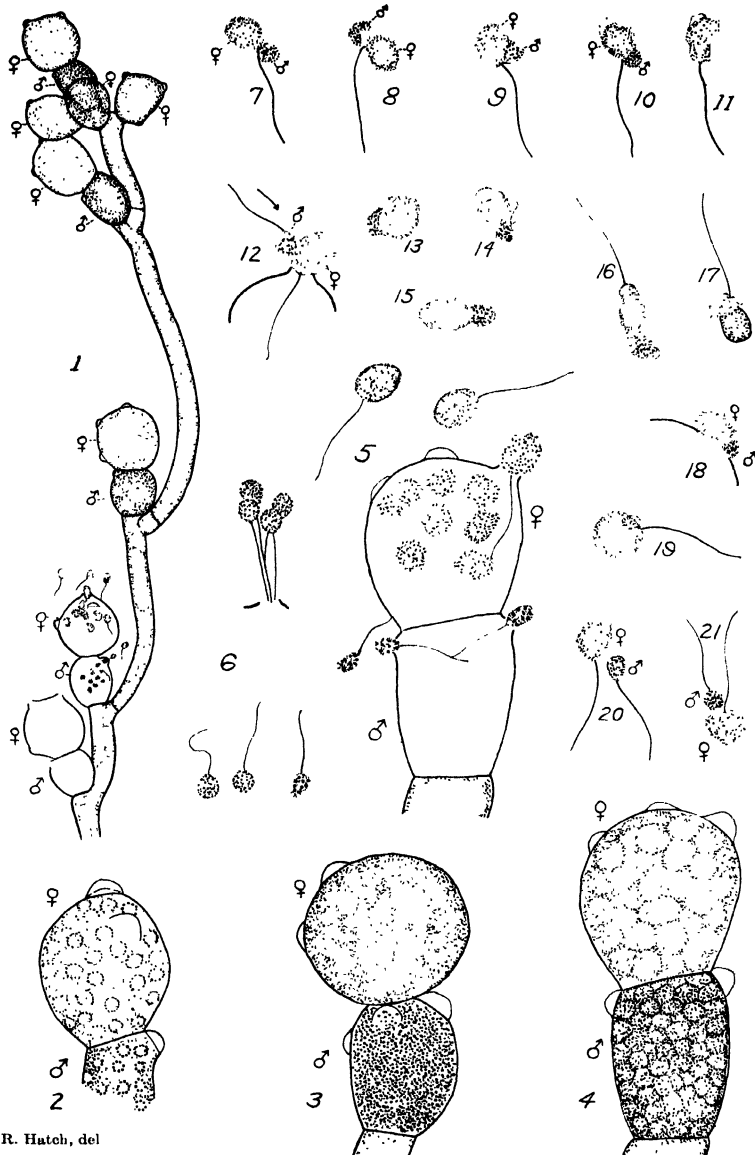
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## EXPLANATION OF PLATE 12

- Fig. 1. Habit sketch of a thread of *Allomyces arbuscula* showing four pairs of male and female gametangia and a cluster of four females and one male at the tip.  $\times 188$ . (This drawing by Dr. John N. Couch.)
- Fig. 2. Gametangia showing vacuoles surrounded by granules, the centers of gamete origins.  $\times 600$ .
- Fig. 3. Disappearance of the vacuoles.  $\times 600$ .
- Fig. 4. Gametes appearing.  $\times 600$ .
- Fig. 5. Gametes emerging.  $\times 600$ .
- Fig. 6. Male gametes.  $\times 600$ .
- Figs. 7-11. Stages in the fusion of gametes. In fig. 11 the male can still be recognized as a minute bulge on the side of the larger female.  $\times 600$ .
- Figs. 12-19. Stages in the fusion of another pair of gametes.  $\times 600$ .
- Figs. 20, 21. Male gametes approaching female.  $\times 600$ .

# PLATE 12



W. R. Hatch, del

*ALLOMYCES ARBUSCULA*



## WIND AND SAND INJURY TO LEAVES AND FRUITS\*

By R. F. POOLE

### PLATES 13 AND 14

Every year wind and sand storms cause much injury to leaves and fruits of many plants. The importance of such injury is not always readily detected immediately after it occurs, since the more prominent symptoms develop sometime later. As a result of the delayed development of the characteristic final symptoms, observers have sometimes failed to clearly diagnose the causes of wind and sand injury when found months afterwards. The velocities of wind and amounts of sand carried in the wind that are necessary to cause either the minor or more serious types of injury were not included in this study. In this treatment of the subject an attempt is made to explain the various types of injury frequently encountered on major agricultural crops.

Injury is not of equal severity every year, indicating that heavy storms of irregular occurrence are responsible for the greatest damage. Major agricultural crops of cotton, tobacco, tomatoes, peaches, melons, apples, grapes, dewberries, and sweet potatoes are affected. Red Bird peach trees transplanted on a southwestern exposure on Norfolk sandy soil in 1931 were partially defoliated by wind and sand storms during the spring of 1932. Leaves on 10-year old Elberta and Georgia Belle trees were injured to a marked degree on similar exposures. Young cotton plants soon after germination were injured so severely as to require replanting over large areas. Tomato and sweet potato plants were also destroyed soon after they were transplanted in unprotected fields. The foliage of tobacco, grape, and many other crops is easily damaged throughout the growing season, but injury is most marked when the wind and sand storms are heavy during the spring season at the time when both the foliage and the fruits are in immature stages of development. Velocities of wind above 20 miles an hour cause the greatest damage to both foliage and fruit. The strong winds also

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carry large particles of sharp sand that exert pronounced puncturing and tearing forces.

Injury of fruit occurs during even mild breezes of sufficient velocity to sway it against limbs or to move leaves with rough margins persistently over its surface. This phenomenon may continue to develop over a period of many days. Roughened leaf margins of dead tissues, caused principally by arsenical acid, are responsible for much minor injury, since fruit hairs and epidermal tissues are partially destroyed. This weakens the tissues, which desiccate to the extent that cracking develops (pl. 14, fig. d). The epidermal tissues are frequently destroyed when the fruit is brought more forcibly in contact with limbs (pl. 13, fig. 1). Injury of both types occurs to a more marked degree when the fruits are small and immature. Gummy excretions develop on peaches when the epidermal tissues are broken (pl. 13, fig. 1). However, this phenomenon is characteristic of injury resulting from any mechanical, chemical, or parasitic disturbance. The injured tissues of green fruits callouse well, but those of matured fruits become much more susceptible to saprophytic fungi, which frequently cause rapid destruction. Spots or slightly sunken areas are produced as shown on the apple fruit when it is pummelled against the ends of dead limbs (pl. 13, fig. 2). This condition is most prominent on trees affected by *Bacillus amylovorus* in previous seasons and on which the dead parts are not pruned out well. The injured tissues develop various shades of brown, apparently due to oxidation.

Leaf injury presents two well demonstrated characters. Tearing by forceful lashings is most prominent as shown on the grape (pl. 14, fig. e) and tobacco (pl. 14, fig. i). Cotton, melons, and various crops are affected in a similar manner. The intercostal areas are partially destroyed, but the veins are successful in perpetuating the life of the leaf. Their strong and fibrous character apparently prevents complete destruction of many leaves. When part of the leaf is so nearly destroyed that the injured parts are unable to function normally, these tissues bleach to such an extent that all green coloring is destroyed (pl. 14, fig. a). A dark brown color occurs in injured tissues, but to a more limited extent than the bleached condition, especially on peach leaves. On cotton, tobacco, and grape leaves the brown color is prominent.

The second character of injury is marked by spots, which result in shot hole characters when the dead tissues shrink and fall out as on grape (pl. 14, fig. e), on tobacco (pl. 14, fig. i), and on peach (pl. 14, fig. o). Similar spots are seen every year on cotton. The spots vary in

size from that of a pin point to more than an inch in diameter. The spots are apparently produced by sand, since spraying peach leaves with coarse limes at 400 pounds' pressure and throwing sand into peach trees produced similar symptoms. Cotton plants from 3 to 4 inches tall when the leaves are small and developing rapidly are often coated with sand particles after heavy rain storms. Even grape and peach leaves high enough to be beyond the reach of sand splashed by water are sometimes coated with sharp grains of quartz, which under force would cut the tissues. A shot hole symptom may occur on any leaf resulting from any cause that destroys the tissues sufficiently to permit the forces of nature to eliminate the dead parts.

The callousing of leaf tissues injured by wind is outstanding. Even when a large portion of the intercostal area is destroyed, the remaining tissues callouse quickly. New epiderm forms quickly on injured tissue as on grape leaves (pl. 14, fig. e) and peach leaves (pl. 14, fig. b). It is interesting to note the ability of the tissue of grape leaves to stimulate greater cell growth, giving a puckered and malformed condition. On peach a prominent toothed character may be noted as shown in (pl. 14 fig. b)—even the margins around the spots callouse (pl. 14, fig. o). Since the injured leaves persist throughout the season, these characters are observed months afterwards (pl. 14, fig. c).

It is inevitable that heavy mechanical injury offers an encouraging source of entrance for certain parasitic and saprophytic organisms commonly found on the various varieties of plants. Fungi found on dead tissues of leaves were species of *Alternaria* on peach and cotton leaves, a species of *Pestalozzia* on grape leaves and species of *Ascochyta* and *Phyllosticta* on cotton leaves, but the common fungous parasites of the various crops were not observed to be any more severe on injured than uninjured leaves. *Rhizopus nigricans* and *Sclerotinia cinerea* did develop more severely on injured ripe peaches than on those that were not injured. Leaf spots on cotton caused by *Bacterium malvacearum* EFS. and on peach caused by *Bacterium pruni* EFS. were worse on injured leaves.

Wooded areas especially the rapidly growing pines on the southwest of fields have demonstrated valuable reduction of wind injury to small crop plants. Growers in the sandhill areas have found it profitable to sow rye in the autumn in fields to be planted in melons, cotton, and other small crops the following spring. Rye is not destroyed at the time of planting the other crops except where the areas sufficient for planting are prepared. Rye reaches two or three feet in height by the

time the cotton plant develops above ground and is left in between the rows of cotton until the latter is of sufficient size and hardness to resist the wind and sand storms. Wind-breaks of evergreen broad leaved plants, used widely in ornamental plantings, are showing effective suppression of wind injury to grape vineyards on the seed farms of Dr. David R. Coker, Hartsville, South Carolina (pl. 14, fig. h). It is clearly seen from these observations that effective wind-breaks may be obtained for both small and large crops at very little cost.

#### SUMMARY

1. Symptoms of injury to leaves and fruits of many plants caused by wind and sand storms are described.

2. Fruits are injured when leaves with dead margins are blown back and forth over the surface and when fruits are pummelled against rough limbs.

3. Leaves are torn in the intercostal areas and on the margins by violent swaying. Spots are also produced by sand, and become shot holes after the injured tissues shrink and fall out.

4. The injured tissues of the leaves callouse well. These ragged leaves persist throughout the remainder of the season even though badly injured early in their development.

5. Injury of leaves resulting from wind damage does not appear to induce in the continuing live tissues a greater susceptibility to fungous parasites and saprophytes. Bacterial parasites on injured cotton and peach leaves were worse. Injured ripened fruits are more susceptible to greater infection and decay.

6. Observations indicate that wind-breaks are valuable and can be grown effectively and economically by using plants well adapted and abundant throughout the South.

#### EXPLANATION OF PLATES

##### PLATE 13

Fig. 1. Type of injury to peaches resulting from being blown against limbs.

Fig. 2. Type of injury to apples resulting from being pummelled against pointed dead twigs.

##### PLATE 14

(a) Bleached symptoms on injured tissues of peach leaves, developing soon after the damage occurred.

(b) Peach leaves showing destruction of margins.

(c) Calloused, toothed tissues observed throughout the season on peach leaves.

(d) Injury to peaches produced by roughened leaf margins.

PLATE 13

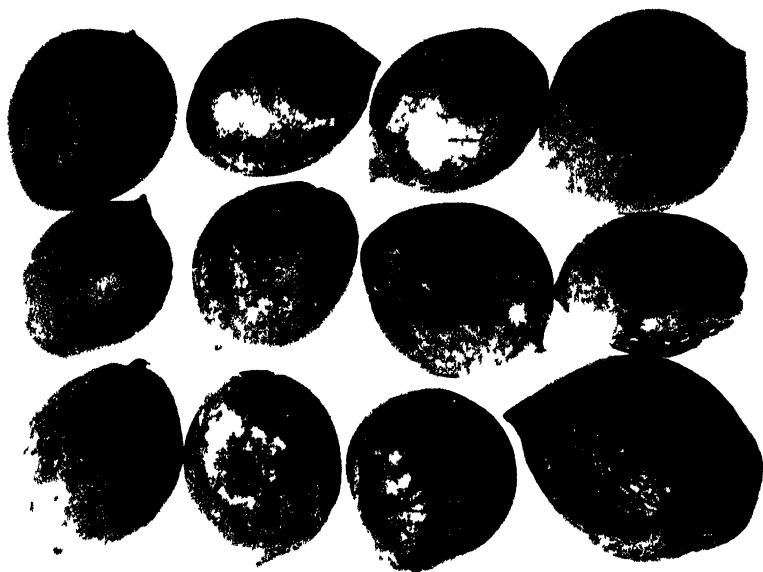






PLATE 14





- (e) Intercostal areas of grape leaves destroyed and calloused puckered tissues formed on remaining tissues leaving a malformed condition.
- (i) Tobacco leaf torn as a result of being lashed by strong winds.
- (o) Small and large shot holes formed in peach leaf after injured tissues shrunk and fell out.
- (h) Effective broad leaved evergreen wind-break on southwest exposure of grape vineyard.

## FATAL POISONING WITH SODIUM NITRITE

By H. B. ARBUCKLE and O. J. THIES, JR.

Sodium nitrite is handled by many textile supply firms, and is distributed without poison labels to cotton mills and dry plants. We have in our laboratory stock room containers from three different manufacturing chemists bearing sodium nitrite labels, but without a poison mark of any kind.

Search of the chemical literature reveals no reported case of fatal poisoning with sodium nitrite in the United States. Two deaths in Bavaria (1), and four in Algeria (2), were attributed to sodium nitrite.

Sodium nitrite is not included in the long list of poisonous substances that the North Carolina Code specifies must be sold under poison label.

Since this substance is used in large quantities in the dye rooms of many mills, we consider it wise to place on record a striking case of fatal poisoning that has occurred in North Carolina.

On October 28, 1932, we were called to investigate the death of the two year old son of L. D. Ray, a worker in a cotton mill in Huntersville, Mecklenburg County, North Carolina.

When we arrived on the scene, the child was dead. His brother directed us to the junk pile near the mill, and stated that the child picked up a small quantity of a white substance, which we saw on the junk pile, and put it in his mouth, and at once ran crying toward his home, which was about 300 feet distant. Before he reached his home, he fell to the ground vomiting. Members of his family hurried to him, and found him pale and weak. On picking him up his limbs hung limp, he was desperately weak, and still vomiting. He was carried directly to his home and the mill physician summoned at once. So rapid was the action of the poison that the child died before the physician arrived. His mother stated that not more than 15 minutes had elapsed since she had picked him up.

With the aid of the physician, a portion of the stomach contents were removed.

The white substance obtained from the junk pile on analysis in the laboratory proved to be sodium nitrite. Examination of the stomach contents showed that they were alkaline and contained a considerable

quantity of sodium nitrite. The solid particles of the stomach contents showed a distinct chocolate brown color. Stomach contents are normally acid, but the hydrolysis of a substance like sodium nitrite would cause the alkalinity. This confirms the detection of a large amount of sodium nitrite in the stomach.

To verify our opinion that sodium nitrite is a violent poison, and was the cause of this child's death, we administered 65 milligrams (1 grain) in a capsule to a cat weighing 2.6 pounds. In five minutes the cat sprawled on the floor, and began screaming. The screaming slowly died down and violent vomiting began. Four minutes later it lost control of its muscles, and in six minutes more, 15 minutes after the dose was given, the cat was dead. This certainly shows the extremely poisonous nature of sodium nitrite.

Chemical literature records the two following cases of fatal nitrite poisoning:

1. H. Molitoris reports two cases of fatal sodium nitrite poisoning occurring within a few months in the same factory, one on October 4th, 1910, and one on April 13, 1911. These were workmen in a factory where sodium nitrite was manufactured in Innsbruck, Austria.

2. L. Musso reports in 1925 four cases of death from sodium nitrite poisoning in a drug store in B., Algeria, by taking sodium nitrite from a bottle labeled sodium tartrate.

The facts presented in this paper show that the use of sodium nitrite as a meat curing agent might be attended with great danger (3).

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# REMOVAL OF MANGANESE FROM PUBLIC WATER SUPPLIES

By E. E. RANDOLPH

## OCCURRENCE AND SOURCES OF MANGANESE IN WATER SUPPLIES

Inasmuch as manganese occurs in some of the public water supplies of North Carolina and because manganese causes considerable trouble in the water system and to some of the industries it was deemed advisable to make a study of methods for removing manganese from public water supplies.

Manganese occurs in small amounts rather widely distributed in the rocks and soils of the state. Manganous salts easily dissolve and enter into the water courses. In at least two or three cases in the state the source of the manganese seems to be in the rock and clay forming the bed of the reservoir.

Organic matter forms  $\text{CO}_2$ ,  $\text{H}_2\text{S}$  and organic acids which tend to dissolve manganese from the rock and clay. These solvent agencies are aided in the solution of manganese by anaerobic bacteria in the bottom of the lake. The oxidation of organic matter in the lake occurs at the expense of the dissolved oxygen in the water. Both of these results produce reducing conditions favorable to the solution of manganese.

## NATURE OF MANGANESE SALTS

Manganese forms two kinds of salts, manganous and manganic salts. Manganese in rocks and in clays as a rule is generally highly oxidized and is not soluble appreciably in pure water, but acids and reducing agents in the water dissolve manganese—first, by reducing it to the manganous form. Since manganese goes into solution in the water by acids and reducing agents in the water, the principles of removing manganese from the water is the reverse, namely, to neutralize the acids in the water and to use oxidizing means to convert the manganese into manganese hydroxide and finally into the  $\text{Mn}_2\text{O}_3$  and  $\text{MnO}_2$ . All practical methods used by large filter plants for manganese removal from water make use of the principle of converting manganese into the insoluble hydroxide and finally to the oxide. The same method applies to iron removal. However, iron is easy to remove because the oxide forms

easily and quickly. Simple aeration is often sufficient for iron removal, but manganese oxide forms slowly, requiring 16 to 24 hours or longer to completely precipitate out.

The oxide does not form unless the pH of the water is high—at least 9 or 9.2 and better at 10 unless oxidizing agent is used. The two essential requirements for manganese removal are high pH and sufficient time.

Both of these conditions are impossible to attain at the ordinary filter plant as the water enters the treating and mixing chamber because the normal plant is constructed to give 20 minutes mixing time and 5 hours settling period. Various devices must, therefore, be used at the average filter plant to remove completely the manganese from the water. In the case that a settling reservoir exists at the plant the water from the impounding reservoir may be made alkaline before it enters the settling reservoir in the plant. Chlorine may then be introduced to oxidize the manganese. The time requirement for the formation and sedimentation for the oxide may thus be met in the settling reservoir. In the construction of the sedimentation basin a concrete bottom should be provided and arrangement made to draw off the sediment regularly.

#### OBJECTIONS TO MANGANESE

1. The manganese in the water is partly precipitated by the lime and dragged down on the filters, clogging up the filters, shortening the filter run, increasing the necessary cost of filtration.

2. Since it takes time for the oxide to form only part of the manganese is deposited on the filters.

3. Its precipitation is aided by heat. Hence heavy coatings of manganese form in the pump and on the steam pipe. This coating not only clogs up the pump, furrows up the cylinders, but greatly cuts down the heat transfer of the steam and clogs up the pipes.

4. Manganese oxide continues to form in the mains and distribution lines, sloughs off when the pH of the water for any reason changes in the system, causing what is known as "black water."

5. Often times when the pH of water is raised to prevent "red water" especially on lines running to dead ends, as the "red water" is mastered it is followed by complaints of "black water," caused by the precipitation of manganese oxide from the water which would have remained in solution in the water at the lower pH and would probably not have been noticed ordinarily.

6. Brownish discoloration of glass, porcelain, and enamel in lavatories.



7. The formation of brown stains on fabrics in laundries.
8. Spotting and staining in sizing and finishing work in textile plants.
9. Streaking and spotting colors in dye plants.
10. A slightly objectionable, or at least a noticeable, taste in the water.

#### PRINCIPLES OF MANGANESE REMOVAL

All successful methods of manganese removal in actual plant practice require the formation of the insoluble hydroxide and the oxide of manganese. Manganese is dissolved as manganous salts from the rock and in the bottom of the reservoir largely by the  $\text{CO}_2$  and the organic acids. Decay of organic matter in water is brought about to a considerable extent by anaerobic bacteria, and the decaying organic matter consumes oxygen (the carbon forming  $\text{CO}_2$  and the hydrogen forming water), and the dissolved oxygen in the water is consumed in the decaying process of the organic matter. As long as an excess of oxygen is present manganese would not likely dissolve because it would be kept in the manganic condition. It is to be observed that the  $\text{CO}_2$  greatly increases, that the oxygen greatly decreases, and correspondingly the manganese greatly increases with the depth of the lake. It is clear that the manganese is stratified in this raw water.

The treatment therefore should be to neutralize the effects of the products formed by the decaying organic matter; for example, neutralize the  $\text{CO}_2$  with lime and add excess lime to bring the pH up above 9, supply oxygen to the water by aeration, give sufficient time for the insoluble manganese oxide to form and settle out before the raw water reaches the regular mixing flume. Aeration of the water will oxidize any iron and help to oxidize any manganese present. Chlorine is a good oxidizing agent and is effective in oxidizing manganese in solution in the water. Potassium permanganate is one of the best oxidizing agents. With its use manganese can be oxidized at a rather low pH.

When copperas or ferric iron salts are used for the floc it is an advantage to take the raw water from the bottom of the lake because the iron in solution in the water is greater near the bottom than at the top and this iron already in the water forms part of the floc material, thus saving the addition of some ferrous or ferric sulphate as floc.

The water at the bottom of the reservoir is cooler, and hence denser than the water at the top. This stratification continues during the period of stagnation and consequently little diffusion occurs so that mineral contents of the lower strata of the water is not equalized throughout the depths of the lake. When, however, the temperature

of the water at the top becomes  $39.2^{\circ}$ , the point of greatest density of water, a turnover occurs in the water and equilibrium is established for a time.

When iron is not used as the floc it is best to draw the water from the top, and if the lower strata can be drawn off into the sewer, diffusion cannot carry so much manganese in the effluent raw water from the lake to the reservoir or mixing chamber.

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## A PHOTOGRAPHIC METHOD OF COLLECTING REFERENCES\*

By THEODORE B. MITCHELL

In the field of insect taxonomy one of the initial difficulties often encountered is the inaccessibility of much of the important literature. This is more than likely to be a very serious difficulty indeed, since in this field a simple review of the literature is entirely insufficient; rather, it is necessary to have a considerable proportion of the published data on any group of insects immediately at hand in order to make satisfactory progress.

Some of the literature, it is true, is quite easily obtainable, since it is often possible to get separate papers or reprints from the authors of recent papers, and separate numbers of serial publications can be frequently purchased. Older papers are usually more difficult to obtain, although they are sometimes copied by the photostat method by libraries or other institutions and furnished at a certain price per page.

However, this haphazard method of collection is rather unsatisfactory, since the supply of separates or reprints is often limited and consequently difficult or impossible to obtain and purchase of separate numbers of periodicals may prove too expensive for the average pocket-book. The photostat method, while absolutely accurate, is very apt to be expensive, unless the equipment is available for the worker to do his own copying, and illustrations copied by this method are very poor, since they are negative rather than positive prints. Moreover, many papers will be practically impossible to obtain by any of these methods, especially references from early publications now out of print and obtainable only at exorbitant prices or not at all.

To facilitate the collection of these references which are otherwise difficult to obtain I have been employing a photographic method which differs from the more familiar photostat process and has several distinct advantages. The equipment used includes principally a miniature camera employing standard 35 mm. motion-picture film. Accessory

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equipment includes a stand for holding the camera in position, a light, and a reflector for use in getting an even distribution of light. A front lens for shortening the focus of the camera is also desirable. The film used in this camera is cut in five or six-foot lengths in the dark-room and rolled into cartridges which can then be loaded into the camera in daylight. It is usually desirable to have six or more of these cartridges where much copy work is to be done without access to a darkroom. For the light, an ordinary desk lamp with a 60 watt bulb has proved quite satisfactory.

With the exception of the lamp, this equipment is very compact, and can be carried in a brief-case or hand bag, and can be used, therefore, in almost any location. While the lamp is somewhat awkward to carry, it is probably a better plan to include such a known source of illumination than to depend upon whatever other sources may be available on the location.

The procedure, briefly, is as follows: The camera is set on the stand at a definite distance above the table level. The material to be copied is placed on the table below the camera, with the light and reflector so arranged as to obtain an even distribution of light, without glare. Time exposures, from 5-10 seconds for the film I have been using, are then made of each page of the reference. Ordinary or contrast film, which is used in making movie positives, is the cheapest to use and is quite satisfactory. Film strips after exposure are developed later, at the office or home; and finally, enlarged prints of each page may then be made at one's leisure and of any desired size.

This procedure has been modified in some degree in preparing a combination bibliography and compilation of references arranged according to taxonomic position in the form of a card index. Enlargements are made on bromide paper cut to 3 x 5 inches, the size of the usual index cards. The bibliographic reference is then typed on the blank side of the print, and this is filed just as any index card, and these are gradually replacing the ordinary index cards in my files. Thus I have not only the reference, but its content on the reverse side, and upon the completion of such a task, I will have assembled for each species a complete set of photographic copies of all the published data pertaining to it. This of course necessitates that an enlargement be made for each species mentioned when two or more are included on the same page of a reference.

There are at least four advantages to be recognized in this method of copying; convenience, speed, economy, and accuracy.

First, as to convenience, the fact that all of the necessary equipment can be easily transported by hand, carried into any institution and utilized to make copies of anything that may to be available and desirable is an outstanding advantage. A dark-room is not immediately necessary, and with a sufficient supply of cartridges for the film, one may work for several hours and copy literally hundreds of pages before it is necessary to develop the strips and reload the cartridges. So far I have had no one raise objections to doing this sort of work in a library or museum, but it is wise of course to get permission from the proper authorities before engaging in such activities if one happens to be a stranger.

The speed with which this work can be accomplished is also a distinct advantage. Even in copying scattered short articles, a strip of 45 exposures can be made within an hour, and a longer paper can be copied even more rapidly, at the rate of at least two pages a minute. Developing the negative strips and making the prints also takes time of course, but can be done more at leisure, at a later time, and at the place which is most suitable.

As to economy, the negative strips made by this method are very inexpensive. Using the contrast film just mentioned the cost per exposure is barely over a fourth of a cent, since the film can be purchased for two cents a running foot and eight exposures are made to the foot. There is a slight amount of wastage at the ends of each strip. The cost of the prints depends of course on the size desired.

This shares with the photostat method the advantage of being absolutely accurate. In the matter of illustrations, however, this method is superior, since a true positive is obtained instead of the unnatural looking negative print obtained with the photostat method.

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**THE BEHAVIOR OF ISOLATED PIECES OF ASCIDIAN (PEROPHORA VIRIDIS) STOLON AS COMPARED WITH  
ORDINARY BUDDING<sup>1,2</sup>**

By ESDA MAY DEVINEY

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<sup>1</sup> A thesis submitted to the Faculty of the University of North Carolina in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Department of Zoology.

<sup>2</sup> The experimental part of this work was carried on in the Biological Laboratory of the United States Bureau of Fisheries at Beaufort, North Carolina, during the summers of 1930, 1931, and 1932. Thanks are expressed to the Commissioner of Fisheries for the privilege of admission to the laboratory, also to the two consecutive directors, Dr. S. F. Hildebrand and Dr. H. F. Prytherch, and members of the laboratory staff for their cordial assistance. Sincerest gratitude is expressed to Dr. H. V. Wilson, under whose direction the work was carried on, for his helpful constructive criticism.

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## INTRODUCTION

The gross structure and general growth habits of the social ascidians have been described by Guard (8), Kowalevsky (10, 11), Ritter (14, 15), Lefevre (12, 13), Brien and Brien-Gavage (2, 3, 4), and many others. *Perophora viridis*, the subject of this investigation, forms colonies of greenish zooids connected by tubular, branching, green stolons which creep over wharf pilings, hydroids, bryozoa, or the tests of other and larger tunicates (fig 1)

In order to obtain data for comparison of the two phenomena, i e , the normal budding method carried on in the establishment of a colony, and the transformation of small pieces of the stolon into zooids, the following technique was employed

Stolons of freshly collected *Perophora*, growing on and among hydroid and bryozoan colonies, were cut into one and two inch lengths and tied on new, clean, glass slides. The slides were then suspended near the top of a battery jar of sea water which was dipped from the surface at rising tide, the water in the jars was changed twice daily. Placing the jars in an aquarium table of running salt water kept the temperature fairly constant. Within forty-eight hours after the material was tied on the slides there were new, healthy, green stolons growing out beyond the original cut ends (fig 1)

Pieces of these new stolons ranging in length from one-half to two millimeters were abscised and placed on slides in finger bowls of filtered sea water which was also dipped from the surface at rising tide. This water was also changed twice daily. The small stolon-pieces were observed and sketched every twelve hours. Transforming pieces of stolon were preserved in Bouin without acetic, in normal Bouin, and in Allen's modification of Bouin. The pieces were stained *in toto* in haemalum, imbedded, sectioned  $5\mu$ , and counter-stained in either acid fuchsin or methylen blue. Freshly collected *Perophora* material showing blastozooids of all ages were treated in the same way and a comparative study was made.

## EARLIER PHASES OF DEVELOPMENT

*a. Establishment of a young colony by the Oozoid*

The normal method of origin of a young colony of *Perophora viridis* was observed under controlled conditions in the laboratory during the course of the investigation. Young oozoids, freshly hatched and about 1-1.3 mm. in length, were removed from the cloacal cavity of the parent zooid, placed on slides in finger bowls of filtered sea water and observed at frequent intervals. The actual time of, and the various steps in the metamorphosis of the young oozoid, or tadpole, are given:

6/17/32, 2:30 p. m., young oozoids were removed from the cloacal cavities of adults which were freshly collected from the harbor. The tadpoles appeared fully formed, but were still inclosed in the egg membrane. 3:00 p. m., tadpoles had become free-swimming and were moving about with a sort of sculling movement of the tail. 5:00 p. m., metamorphosis had begun; the sensory vesicle had decreased in size, and the larval sense organs had disintegrated; the notochord was an amorphous mass at the base of the tail; the adhesive papillae had elongated; the heart was observed beating, and streams of blood could be seen passing in and out of the two ends of the heart. 6/18, 9:00 a. m., metamorphosis was complete: branchial stigmata and siphons were present. There was no sign of either a tail or a notochord. Only a small remnant of the sensory vesicle remained—between the siphons. The outgrowths from the adhesive papillae had become definitive stolons, four in number. Two of these stolons were green in color and were beginning to attach to the slides on the bottom of the dish. 6/22, only two of the original four stolonitic out-growths seemed to be flourishing. 6/28, only one healthy stolon remained. This bore a young bud or blastozooid about 2 mm. from the oozoid.

Except for some details the metamorphosis from tadpole to adult oozoid is well known. When the first bud has been formed successively younger ones appear nearer the tip as it grows forwards on the substratum. The growing stolons give rise to side branches which also bear buds; thus are formed complex colonies in which it is soon impossible to distinguish the original oozoid and its original stolonitic out-growths.

This account of the establishment and the morphology of a colony agrees with that of Brien and Brien-Gavage (4) for *Perophora listeri*, but differs very strikingly in respect to morphology from a species of the Pacific coast, *Perophora annectens*, as described by Ritter (14). The



latter says that the Pacific coast species may be incrusting, i.e., very flattened, and that the individuals and stolons may be enveloped in a common tunic. In the possession of simple linear stolons on which are borne the blastozoids of varying ages and sizes, *Perophora viridis* and *P. listeri* both differ in morphology from *Clavelina lepadiformis* as described by Brien and Brien-Gavage (3). "Clavelina sends out from the base of the post-abdomen radial stolons which are solidly fixed to the support. Each stolon branch generally presents at its distal end a dilatation which soon detaches (from the ascidian) and becomes the blastogenetic center at the expense of which a blastozoid is formed. Blastozoids of the same cluster have no direct or physiological connection with each other. They do not form true colonies."

#### *b. General structure of the normal stolon*

The structure of the stolon is not so simple and definite as some of the earlier accounts describe it. The wall of this tubular structure really consists of three layers of tissue: (1) an outside tunic with scattered cells and much intercellular substance, (2) a thin epithelio-ectodermal layer in which cell boundaries are, with the technique employed, not visible in sections, and (3) a delicate layer of mesenchyme cells, interconnecting often, forming a discontinuous lining of the stolon cavity. This cavity is divided vertically by a thin single-layered septum attached at both edges to the ectodermal layer of the wall and continuous with bits of the mesenchymal lining which chance to lie along the line of attachment (fig. 2). Right and left halves of the stolon cavity communicate at the distal tip, as the septum is lacking for a distance of one-fourth to one-half millimeter at this point. The origin of the septum of *Perophora viridis* was not ascertained in this study, but Brien and Brien-Gavage (3, 4) state that it is mesenchymatous in origin both in *Clavelina lepadiformis* and in *Perophora listeri*. They describe how it is formed by the fusion of the wall of two blood sinuses growing out from the body of the oozoid. The connections between the blastozoid and the stolon are found here, as in Brien's investigation, quite like those existing between the oozoid and the stolon. A discussion of this point is given later.

#### *c. Cellular components of the stolon*

Sections were prepared with the technique mentioned above and the living stolon unstained and after staining with weak (1:10,000) neutral

red was repeatedly studied. Examination with the immersion objective and a reasonably high ocular is necessary.

*Observations on the living stolon.* In the living stolon stained with neutral red the cell most in evidence in the tunic was a large amoeboid body containing six to eight dancing, red granules (fig. 3). This is undoubtedly the compartmental amoeboid cell described by George (6) although the "compartments" could not be seen with the technique employed (George employed for this purpose another technique). These cells are listed among the free blood cells of the haemocoel by George, but in this investigation they could not be observed in the haemocoel of the living unbroken stolon. However, a living stolon whose ectoderm and tunic were punctured before being stained with weak neutral red, showed these cells with the red granules among the blood cells which streamed out of the punctured place.

In an instance or two some large, pale amoeboid cells were observed creeping into the tunic from the haemocoel through the ectodermal cells on the distal end of the stolon. These were probably of the compartmental amoeboid type, but since the specimen under observation at the time was not stained no granules were to be seen. Possibly these cells become tunic cells. If so, the observation in a measure confirms Brien's conclusions as to the origin of the tunic cells. Brien (2) says that, in a detunicated piece of *Clavelina* stolon, the presence of ectoderm is necessary to the reformation of the tunic. However, he believes that the actual tunic substance may be borne by migrating, vacuolated, mesenchymal cells which wander out of the haemocoel through the ectoderm and they, upon contact with water, yield the substance which forms the new tunic.

In the living stolon the ectoderm at the tip is seen to be thick (fig. 3); elsewhere it is thin. The boundaries of tall columnar cells are sometimes faintly visible where it is thick. Within the haemocoel or stolon cavity the septum may be seen as a thin, vertical plate in the median line. The free, circulating blood cells which are easily seen in the haemocoel of the unbroken stolon are, in the terminology of George, green cells, morula cells, signet-ring cells, and orange cells, and some pale colorless types which are presumably lymphocytes. The nuclei are not visible in any of these living circulating cells. A green cell and a morula cell are shown in figs. 6a and 7a respectively. There is no need to describe these cells in the living state as this has been done in admirable manner by George (6, 7), but it is necessary to identify the several types in the fixed material and every effort has been made to do this.

*Observations on fixed material.* Not all the cells of the sectioned, stained material were easily identified with types of cells of the living stolon. In the tunic of the fixed material the most abundant cell is an irregularly amoeboid body with a fair sized reticulate nucleus which is more often elongated than spherical in form. The range in size of these cells is: length, 7-13 $\mu$ ; width, 3-6 $\mu$ ; nucleus, 3-4 x 2-2 $\frac{1}{2}$  $\mu$ , or if spherical, 3-3 $\frac{1}{2}$  $\mu$  in diameter (fig. 4, b & c). These sizes are close to those of the tunic cells with dancing granules seen in the living stolon. Often several of these cells are seen to be connected by cytoplasmic strands, or they may be isolated, in which case they may or may not show pseudopodial protrusions of their cytoplasm. Very rarely they form a layer, almost epithelial, at the inner wall of the tunic next to the ectoderm. These cells of the fixed material are doubtless the cells with dancing granules, that is, the compartmental amoeboid cells of George (6). Proof lies, however, only in their abundance, size, and position since the granules are not seen in the preserved cell nor the nucleus in the living cell. The compartments are noticeable in the fixed material only in rare cases. George states that a special method of treatment is necessary to bring out this feature in the living cell. Absence of the granules in the preserved form is probably due to their dissolution by the fixative. Bodies which possibly correspond to these granules are recorded by Brien (2) as present in the cells which pass out of the haemocoel to give rise to the tunic. Brien was able to preserve these inclusions by the use of Bouin without acetic acid. This method failed to preserve the granules in the tunic cells of *Perophora*.

Another type of cell found in the tunic of the fixed material is a rather smooth amoeboid form containing a deeply staining homogeneous body which is probably the nucleus (degenerating?) since no other nucleus is to be seen (fig. 5a, b, c).

In the fixed, stained material, the ectodermal cells at the distal end of the stolon clearly show their columnar structure; elsewhere they are flat and thin. Cell boundaries are never very distinct in the ectoderm at any point. The oval nuclei of the ectodermal cells are poor in chromatin, most of which is usually confined to a nucleolus and perhaps a scattered particle or two. Very rarely there are two small chromatin masses of approximately equal size. A distinct nuclear network cannot be made out.

The septal cells are exceedingly thin and flat in the non-budding region, less so in the budding region. No cell boundaries are visible in the septum at any time, either in sectional view (fig. 2) or in face view.

The oval nuclei resemble the nuclei of the ectodermal cells in the paucity and arrangement of the chromatin. In their staining reactions, also, the ectodermal and septal cells are very similar.

In both the preserved and living material there are three types of free blood cells in the haemocoel which are easily identified with George's cells. These are the green cells, morula cells, and signet-ring cells. The abundant green cells present practically the same appearance in both the living and the fixed stained material, at any rate they retain the green coloring and vacuolated appearance. The nucleus in the fixed cells may be seen as a slightly eccentric, reticulate, vesicular body lying in a mass of homogeneous cytoplasm from which radiate cytoplasmic strands between the several vacuoles of the cell (fig. 6b, c). The cells measure from 7 to  $10\mu$  in diameter.

The morula cells in their appearance suggest early stages of segmenting eggs. Their subdivisions rarely exceed 4 to 6 in number. Single, small, reticulate, oval nuclei may occasionally be seen in material which has been destained sufficiently (fig. 7b & c). The range in size of these cells is about  $6 \times 11\mu$  to  $9 \times 12\mu$ .

Only an occasional signet-ring cell is found. These cells have a size range about like that of the green cells. There is a fair sized reticulate nucleus in the thicker part of the rim of cytoplasm which surrounds the large eccentrically placed vacuole (fig. 8). The orange cells of the living stolon are not identifiable in the sectioned material.

One very abundant type of spherical cell which stains a brilliant cerise with acid fuchsin can probably be identified with George's granular amoebocyte. This cell is packed with spherules which seem to vary in density and which very often obscure the small solidly-staining nucleus. The diameter of this cell is always around  $9\mu$  and that of its nucleus from  $2\frac{1}{2}$  to  $3\mu$ . Under certain physiological conditions this cell stains a mulberry red instead of cerise, but it is always easy to identify by its spherules (or granules) and by its constant size and form (fig. 9).

One type of free cell in the haemocoel closely resembles the most common tunic cell. Its cytoplasm is apparently homogeneous and it usually stains very lightly. The nucleus is reticulate and of the same average size as that of the tunic cell which has been designated the compartmental amoeboid cell. This cell is usually a bit more rounded in form when found in the haemocoel (fig. 4a). It may occasionally be seen making cytoplasmic connections with others of its kind and even forming small syncytia. Very often it is found closely applied to the ectoderm through which it might easily migrate.

Finally, for this investigation, the most interesting and important type of cell in the haemocoel is a small lymphocyte with a scant amount of finely granular cytoplasm surrounding a relatively large nucleolate nucleus (fig. 10 a-d). These cells measure from 5 to  $8\mu$  in diameter. When rounded in form their average diameter is  $6\mu$ . The nuclei measure 3 to  $6\mu$  in diameter, the average size being  $4\mu$ . These cells are almost always spherical although one is occasionally seen putting out a blunt cytoplasmic process. In addition to the nucleolus there are often scattered bits of chromatin in the nucleus, more of them than are to be found in the nuclei of the ectodermal and septal cells. Rarely there is found a lymphocyte in which instead of a nucleolus there are scattered chromatin particles looking as if they might be parts of a reticulum or nuclear network (fig. 10d). In their most simplified form (fig. 10a) these cells look very much like the figures of the lymphocyte type described by Brien and Brien-Gavage (4) for *Perophora listeri*, and again like those of a Bermuda ascidian, *Symplegma viride*, described by George (7). On the contrary George's description of the lymphocyte of *Perophora viridis* shows it to be more like the type in figure 10d which may possibly be regarded as an early transition stage between the primitive lymphocyte and some one of the more specialized forms, e.g., the tunic cell.

Cells resembling lymphocytes may often be seen applied to the septum, or to the ectoderm (fig. 2). Similar cells with processes (fig. 11a, b, c) often interconnecting with one another occur in the haemocoel. These are presumably homologous with the mesenchymal cells of Brien and Brien-Gavage (3, 4) which they say line the entire haemocoel in *Clavelina lepadiformis* and *Perophora listeri*. In *P. viridis* they do not make a continuous lining, but form a discontinuous layer just inside the ectoderm, and they occasionally are found free in the haemocoel as if in migration. Brien (2) states that these and the lymphocytes are different stages of the same element, a conclusion which appears probable.

The above account of the normal structure of the stolon of *Perophora viridis* differs in several details from Lefevre's (13) description. He considers the entire septum as a "collapsed cylinder the walls of which are pressed closely together and attached along the upper and lower borders to the inner surface of the ectodermal tube." Lefevre does not distinguish between the different types of free blood cells. Otherwise his account is correct. Kowalevsky (10) likewise describes the septum of *P. listeri* as being formed of two layers of cells. On the

contrary, Brien and Brien-Gavage (4) state that in *P. listeri* the septum is single everywhere except in actively budding regions. This is the condition in *P. viridis*, and the point is an important one in morphogeny. As to the method and place of origin of the septum in *P. viridis*, this has never been definitely worked out. Lefevre (13) threw doubt on its endodermic, epicardiac origin, a view held before his time from the general likeness of this species to the Clavelinas. Van Beneden and Julin (19) had previously stated that the septum of the stolon of *Clavelina* had its origin in the epicardium, an outgrowth from the median wall of the pharynx above the heart. Lefevre found no epicardium in *Perophora*, but was unable to get oozoids of *Perophora* to transform sufficiently to see the origin of the septum. Brien and Brien-Gavage (3, 4) have found the septa of stolons of both *Clavelina lepadiformis* and *Perophora listeri* to have a mesenchymatous origin, to which account reference has already been made.

In their classification of free mesenchymatous or blood cell types of *P. listeri*, Brien and Brien-Gavage (4) have described homologues of certain of the above blood cells of *P. viridis* as described by George (6, 7), viz., green cells, signet-ring cells, orange cells, and lymphocytes, but they have not been able to establish identity between all the blood cells of the two forms. The three authors, however, agree that the lymphocyte is the most primitive type of blood cell and that transition stages between this and the more specialized types can be found. Brien and Brien-Gavage (4) have figured such transition forms between the lymphocyte type and all the more specialized blood cells of *Perophora listeri*. This has not been done for *P. viridis*, although George (7) has found in another genus, *Ecteinascidia*, what he believes to be intermediate stages in the formation of some of the highly specialized types of blood cells.

#### NORMAL BUDDING

New young buds or blastozoids, of the *Perophora* colony, are almost invariably formed between the smallest visible one already formed and the growing tip of the stolon. Many stolon tips with incipient budding stages were examined, first *in toto*, then as serial sections. No differences of any importance were observed, in the normal budding process, between material freshly collected from the outside and that grown on slides in the laboratory as described above.

For convenience of description the budding process may be divided

into three stages: (1) the formation of a small vesicle from the upper part of the septum, a stage not usually visible externally; (2) the growth of this vesicle and the accompanying formation of certain organ primordia, this stage marked by a hemispherical protrusion of the upper surface of the stolon; (3) the growth and differentiation of the organ primordia. The last stage is visibly accompanied by a great increase in size of the bud, by its constriction from the stolon, by its inclination toward the stolon tip, and, finally, by the assumption of activities such as heart beat, contraction and expansion of the siphons. Naturally, hard and fast lines cannot be drawn between these stages.

*First stage in development of bud.* The following description of the first stage is based on the study of a series of transverse sections of a stolon which was collected and preserved immediately in Allen's modification of Bouin.

The tunic varies in thickness. It may be very thin. At the distal end of the stolon it is about  $21\mu$  thick. The ectoderm at this end is columnar, as said, and about  $10\mu$  thick. In both cross and longitudinal sections the cells appear to be more or less loosely joined together, i.e., they are marked off from one another by vague light streaks. Such an arrangement would be well suited for the migration of cells from haemocoel into tunic. Back of the tip the ectodermal cells become thinner and finally reach their attenuated form within a distance of about  $100\mu$ . For a distance of about  $200\mu$  the ectodermal wall incloses an undivided cavity, the haemocoel, circular in cross section, whose only contents are a few scattered blood cells; there is no sign of a septum.

At a point  $230\mu$  back of the stolon tip the septum appears, in the midst of the free blood cells of the haemocoel, as an irregularly polygonal mass of cells representing the end of a rod or plug (fig. 13). Within the distance of  $14\mu$  this rod becomes a one-layered plate of cells which soon ( $15\mu$ ) becomes two-layered in its upper part (fig. 14). It is noticeable and remarkable that no cell boundaries are visible in this mass, and yet, with the technique employed, one would expect them to be visible if there. Immediately ( $7\mu$ ) back of this point, the two-layered upper part of the septum acquires a cavity, while its undivided lower part shows, in section, two rows of nuclei in the thickened cytoplasm (fig. 15). Behind\* this point the cavity extends ventrally through the greater part of the septum, until, at a distance of  $70\mu$  from the beginning of the cavity, the section shows an egg-shaped vesicle  $40\mu$  wide and  $64\mu$  high. The larger part of the vesicle is directed toward the upper, unattached side of the stolon, while its lower, smaller part joins the small

remnant of the stolonial septum here consisting of a single layer of cells (fig. 16). Proximally, that is, toward the ascidiozooid from which the stolon grew out, the vesicle which is the inner or endoblastic vesicle of a young bud, gradually decreases in size, until, at a distance of  $200\mu$  from its origin it comes to an end as a vesicle. At this point it passes into the septum which here has the same form as it has just distal to the vesicle, i.e., thickened above and one-layered below (fig. 14). Thirty-five micra proximal to the vesicle the whole septum becomes a one-layered plate of cells which gradually (in a distance of  $308\mu$ ) becomes extended entirely across the haemocoel. This condition persists for the distance of 1 mm. At the end of this distance a well developed bud is found attached to the stolon.

The above description is based, as said, on the study of a stolon collected in the harbor. Similar studies were made of stolons grown on glass in the laboratory. There were no differences of interest. To the description as given certain minor additions may be made, some observed in stolons grown in nature, some on those grown in the laboratory. In the first place the nuclei of the blastogenetic part of the septum (figs. 13-16) are characteristic septal nuclei, i.e., they are oval and nucleolate, although slightly larger than nuclei of the septum in a non-budding region. Again the vesicular swelling may at the middle level of the vesicle involve the entire septum (fig. 17a), the remnant of the septum shown in fig. 16 disappearing as it too becomes vesicular. Again there are certain irregular eminences on the outer wall of the young endoblastic vesicle which have some interest and to which return is made later. And also the septum in the immediate neighborhood of an endoblastic vesicle may show certain extensions prolonged out into the haemocoel as in fig. 18. Such eminences and extensions at first sight would be thought of as proliferations of the septal tissue. But there are some facts which lead to the idea that they really represent cell masses of the haemocoel which have united with the septum.

Brien and Brien-Gavage (4) have noted similar projections from the septum of *Perophora listeri*. They regard such spots as places of attachment of free mesenchymal cells of the haemocoel, and say that they furnish further proof of the mesenchymatous origin of the septum.

This question of the participation of free blood cells in the formation of the blastogenetic septum is taken up later.

While the above account of the origin and early appearance of the bud anlage agrees essentially with the accounts of Kowalevsky (10), Lefevre (13), and Brien and Brien-Gavage (4), there are some differences espe-



cially from the findings of the first two investigators. Kowalevsky finds the inner vesicle of the bud arising as a lateral evagination of one of the two layers of a permanently double septum. This, of course, would make the buds appear laterally, i.e., to left or right of the septum, instead of dorsally on the stolon, and his figures show this lateral location. Lefevre, also, considers the septum as everywhere and permanently two-layered, and of a very constant and regular nature, which is obviously not the case. He, however, shows the bud protruding dorsally from the stolon—the correct position. Brien and Brien-Gavage agree with Lefevre except in the matter of the structure of the septum. This they find to be of mesenchymatous origin, and, except in the budding region, one-layered.

In respect to the structure of the septum the results of this investigation are in general agreement with those of Brien and Brien-Gavage (4). The idea held by Kowalevsky and Lefevre that the septum is a two-layered sac was in all probability based on the study of blastogenetic parts of the septum. Sections of the septum in such places, e.g., fig. 17a, may be quite like the sections figured, especially by Lefevre (13).

The growth changes involved in the formation of the inner vesicle of a young bud are of interest.

(1) As has been said, the septum in its apical region (figs. 13, 14) does not extend from ectoderm to ectoderm across the haemocoel as it does elsewhere. This is to be expected since we are dealing with a growing end. The free end is to be looked on as the earlier state. With nuclear proliferation and growth the plate-like state is reached.

(2) Cell multiplication undoubtedly occurs in the septum of a budding region, since mitotic figures may be seen. But while the growth in such a region is active, the mitotic figures are few. There are some suggestions that amitosis occurs. At any rate many nuclei are observed which contain two distinct masses of chromatin of equal size and often such nuclei have a constriction in the middle region.

(3) Finally, there are many indications that lymphocytes and the mesenchymal cells, scarcely to be distinguished from them, are added to the septum. Thus in fig. 19 are to be seen two such cells in contact with the septum and another cell which has already established cytoplasmic continuity with the septum. And many such instances are found. Moreover, there are to be found little plates of cells (fig. 17b) in the haemocoel in the region of the young bud, but not elsewhere, and in these the nuclei and cytoplasm have the same appearance as in the lymphocytes. The irregular projections already mentioned as

occurring on the septum, still in its plate-like condition (fig. 18), and on the wall of the endoblastic vesicle are presumably to be looked on as formed by the coalescence of such little plates with the septum or vesicle respectively. Thus in the transformation of the plate-like septum into a vesicle, while intrinsic growth, possibly with some amitosis, plays the more important part, it would seem that the amount of available substance is also increased by the incorporation of free lymph cells or of little masses formed by the fusion of such. This view is in general accord with that of Brien and Brien-Gavage (3, 4).

*Enlargement of the endoblastic vesicle and formation of a protruding bud.* One of the earliest steps in this phase of the development consists in a thickening of the wall of the endoblastic vesicle and of the ectoderm dorsal to it.

As respects the growth of the vesicular wall it is found that mitoses are especially numerous in it at this stage and it may therefore be concluded that intrinsic growth is going on. Also there are microscopic pictures which indicate that, as at an earlier stage, lymphocytes continue to fuse with it. As to the dorsal thickening of ectoderm, mitotic figures are not found in this region, but again pictures which look like stages in amitosis occur with frequency.

Following on this increase in thickness of the vesicular wall in general and of the ectoderm dorsal to it comes the stage usually referred to as the protruding bud. Among the growth processes involved in this change, it may be noticed that the localized thickening of ectoderm has disappeared. It may possibly be looked on as a preliminary nutritive state preparatory to the establishment of a greater area of comparatively thin ectoderm. The increase in size of the endoblastic vesicle is doubtless, as said, brought about chiefly by its intrinsic growth. In its protrusion the ventral septal remnant, shown in fig. 16, seems to be a causal factor. Comparison of section figures of the earlier stage (fig. 16) and the stage of the just protruding bud shows that this plate-like remnant thins out and lengthens in a dorso-ventral direction. The effect of this would be to push the endoblastic vesicle dorsally. At this stage there is no special change in the thickness of the vesicular wall. As already said, there are buds in which the entire septum is locally transformed into a vesicle. In such cases the causal factor just alluded to would apparently not exist. Lefevre (13) has discussed this question.

As the young bud grows dorsally, it begins to constrict off from the stolon laterally and to incline toward the free tip of the stolon. In this stage, then, the young bud forms a more or less spherical protrusion on

the dorsal wall of the stolon usually about one and a half to two millimeters from the stolon tip. In its gross structure such a bud consists of two vesicles, an inner formed by the stolon septum with which it is still connected, and an outer vesicle formed by the stolon ectoderm; a tunic layer, continuous with the stolon tunic, covers the bud. Between the inner and outer vesicles of the bud there is a cavity, the haemocoel which is in direct communication with the haemocoelic cavities of the stolon. Thus free blood cells of the latter cavities have access to the haemocoel of the bud.

*Organogenetic processes in the bud.* In its organogenetic processes the young bud appears to follow rather closely the method described by Lefevre (13). In brief, Lefevre found that the inner or endoblastic vesicle of the bud formed the peribranchial chambers and cloaca, as well as the pharynx and entire digestive tract, while free blood cells formed the nervous system (dorsal tube and ganglion), pericardium (and heart) and the gonads.

In a bud of about the same age as the one described above there may be found on the dorsal wall of the endoblastic vesicle near its anterior end, i.e., the end directed toward the distal end of the stolon, a small aggregation of lymphocytes. This aggregation, in the cross-sections, first appears in a linear shape, but later takes the appearance of a solid, rounded mass which is applied rather closely to the dorsal wall of the vesicle, i.e., the primordium changes from a loose sheet of cells to a solid cord lying anteriorly in the median line on the dorsal side of the inner vesicle, and lying posteriorly a bit to the left of the median line. From this mass of cells, and possibly other additional lymphocytes which subsequently join it, are formed the hypophyseal organ and the ganglionic mass dorsal to it. At no time have cells from the dorsal wall of the endoblastic vesicle been observed in the act of passing into this mass, although such an action would not be impossible to conceive, since cells of both organs seem to be identical in structure and perhaps in origin.

In buds thus exhibiting the primordium of the nervous system there can be found more posteriorly, near the center of the right side of the endoblastic vesicle, a similar, but perhaps slightly larger, solid aggregate of lymphocytes, the primordium of the pericardium (and heart). This mass subsequently acquires a cavity and the surface turned toward the inner vesicle invaginates to form the heart. As in the case of the anlage of the nervous system there seems to be no evidence that cells enter the pericardial mass from any source other than the blood stream,

although this anlage, too, is rather closely applied against the wall of the inner vesicle after the aggregation has reached considerable size. Both primordia, the posterior end of the nerve-hypophyseal and the anterior end of the pericardium, are shown in the transverse section, fig. 20. The nerve-hypophyseal primordium is again shown at a higher magnification in fig. 21, the pericardial primordium at a higher magnification in fig. 22.

Shortly after the cell aggregates constituting the primordia of the nervous system and pericardium have reached a compact solid form, the middle and posterior regions of the endoblastic vesicle undergo a rotation of about 90 degrees. Lefevre (13), the first accurately to describe this action, says that the rotation is brought about by the thinning of the wall of the vesicle except on the right side. The present investigation verifies Lefevre's findings. The rotation results in bringing the thickened right side of the vesicle to a ventral position, and in shoving the connection of the septum and former ventral wall of the vesicle to the left ventro-lateral region. The thickened region now brought ventrally subsequently becomes the endostyle.

The rotation of the inner vesicle also affects the position of the pericardial and dorsal tube primordia which are at this stage rather closely pressed against the vesicle. The pericardium is now pulled down to a position on the right ventro-lateral region of the vesicle where it remains permanently. The middle region and posterior end of the primordium of the nervous system are brought to a mid-dorsal position. It will be recalled that the anterior end of this anlage forms in approximately the median dorsal line. The position of this portion is not disturbed since the rotation affects the anterior end of the vesicle very little.

Concurrently with the rotation of the inner vesicle there appear in its latero-ventral wall two parallel, longitudinal furrows, one on each side of the future mid-ventral line, which press dorsally and slightly mesially so that their inner walls meet before touching the dorsal wall of the vesicle. Thus the original endoblastic vesicle is divided into an upper and outer part, crescentic in cross section, which becomes cloaca and peribranchial chamber, and a lower median vesicle which becomes the pharynx. As Lefevre (13) points out, at first the two sides of the peribranchial chamber are asymmetric; the right side extends farther anteriorly, while the left side extends farther posteriorly. As development proceeds these differences are eliminated, and the lateral, anterior, and posterior borders of the outer vesicle are extended to surround the pharynx. However, the extreme anterior and posterior ends and ven-

tral side of the latter are never covered entirely by the outer vesicle. The pharyngeal and peripharyngeal cavities are put into communication later by the formation of a series of branchial stigmata.

About the time the peribranchial chambers and cloaca are being separated from the pharynx, there grows out from the left, dorsal, posterior wall of the latter a small tubular evagination. This tube, which is the primordium of the digestive tract, elongates and curves, first ventrally, then anteriorly, and finally dorsally, until it reaches the cloacal wall. In the meantime the tube has differentiated into three regions, (1) a slender sharply curved esophagus, (2) an enlarged stomach, and (3) an intestine which is longer than the other two regions combined and which itself has two enlargements near its origin. Sometime later an opening is formed at the point of contact between the intestine and the cloacal wall. Later, also, there arises from the wall of the stomach a slender evagination, the "organe refringent" which branches many times, each branch finally ends in an enlargement, ampulla, pressed against the intestinal wall.

While these developments have proceeded in the digestive tract, the pharynx and peribranchial chambers have been establishing communication by the formation of branchial stigmata. The latter seem to form according to the method described by Lefevre (13). Thickened spots appear first in the pharyngeal wall. These spots are evaginated slightly to touch the inner wall of the peribranchial chambers and this invokes a thickening of the latter walls at the point of contact. The two walls fuse where contact is made and an opening is formed at the point of fusion. However, the first thickening occasionally appears in the wall of the peribranchial chamber as Beers (1) has described for *Ecteinascidia*.

While the earliest stigmata are being formed the primordia of the oral and atrial siphons can be seen in the process of formation. These, too, follow the same course as described by Lefevre. Ectodermal invaginations occur, one over the anterior end of the pharynx and one over the middle of the cloaca. Where these touch the pharyngeal and cloacal walls the latter are thickened slightly. Later an opening breaks through at the bottom of each invagination. Around the invaginations for both siphons many mesenchymal cells aggregate. These form the chief muscles of the body, i.e., those which control the siphons.

Connections between stolon and blastozoid change considerably as the latter grows. At first, the young bud lies at right angles to the stolon, and the haemocoelic cavity of the bud opens widely on each side into the corresponding half of the haemocoele of the stolon (fig. 20).

By the rotation of the endoblastic vesicle its connection with the septum is shoved to the left. As the peribranchial furrows are formed the left one is placed mesially to the connection of the septum and the vesicle so that the septum remains in connection with the left peribranchial primordium. The latter connection lasts for a time, but is broken during the further growth of the bud. In this growth the bud becomes inclined toward the tip of the stolon and is moreover more distinctly constricted off from the stolon. As the zooid increases in size and assumes activities, this narrowed connection with the stolon increases in length and assumes the aspect of a stalk. The stalk is finally divided into two at the point of exit from the zooid, and these two, *a* and *b*, are shown in transverse section in fig. 24*a* and *b*. In the region of connection with the stolon the stalk remains undivided and is shown in transverse section in fig. 25. The stalk divided or undivided, contains, of course, the haemocoel with free blood cells, figs. 24 and 25. At this time some of the haemocoelic cells within the blastozooid unite, as Brien and Brien-Gavage (4) describe, to form the walls of two sinuses. These sinuses they designate subendostylar and subintestinal. The former is directly continuous, as Brien and Brien-Gavage say, with the heart, the latter is said to be indirectly connected with the heart through the perivisceral network of sinuses. The two sinuses are shown in fig. 23 which represents a part of a section through a young blastozooid. These two sinuses are extended into the two divisions of the stalk-like connection between zooid and stolon. In fig. 24*b* the imperfectly formed wall of one of the sinuses is shown, while in the other division of the stalk, *a*, the sinus formation has not begun. In the undivided part of the stalk the walls of the two sinuses come in contact and partially fuse as is shown in the transverse section in fig. 25. This region of fusion is continuous with the septum of the stolon. The two sinuses are continuous with the two haemocoelic cavities of the stolon, one on each side of the septum. The septum is thus seen to be quite continuous with mesenchymal structures of the blastozooid, and as already stated, Brien and Brien-Gavage (4) find that in the oozoid it originates as a mesenchymal structure.

No mention has yet been made of the gonads. The early common primordium of these is to be seen as a group of mesenchymal cells in the loop of the intestine about the time the latter becomes fully differentiated. The transformation of this primordium into the gonads and their ducts has not been followed. Lefevre (13) has covered this ground.

Only a brief account of organogenesis has been given since the various

steps have been found to agree in nearly all details with Lefevre's description (13). This short account will serve as a basis for comparison between the processes as they occur in normal budding and in the abscised pieces.

#### ISOLATED PIECES OF STOLON

##### *Early behavior of the longer pieces*

The stolons of the *Perophora* stock-material, which was grown in the laboratory in a previously described manner, appeared normal in structure and budding activities. Hence it was assumed that isolated pieces of such laboratory-cultured stolons would act in essentially the same way as isolated pieces of the natural, freshly collected stolons. The latter would have been much more difficult to isolate. Accordingly, loose, unattached ends of the stolons, which projected conveniently beyond the material originally tied on the slides, were cut off, sectioned into small pieces ranging from one-half to two millimeters in length, and placed in finger bowls of sea water which was carried in from the outside and filtered.

Within one to three hours after being cut, all pieces seemed to be healed at each end and to have shrunk slightly within the tunic which had contracted and covered the cut surfaces. Often, within 24 hours after being cut, the ends of the pieces had extended outside the bounds of the old tunic and had secreted a delicate new tunic layer. Sometimes the entire piece withdrew from the old tunic and secreted a new tunic around itself.

In general it may be said that the longer pieces, i.e., those of more than one millimeter in length, usually elongated slightly and budded in apparently normal fashion. The majority of such buds were placed normally on the stolon piece; however, an occasional case of terminal budding occurred in these pieces. Brien and Brien-Gavage (2, 3) found terminal budding to be the rule and not the exception in isolated bits of *Clavelina* stolon. Some of the longer pieces of *Perophora* stolon showed signs of budding very shortly after isolation and before any noticeable elongation had taken place. These pieces probably came from incipient budding regions which had their activities only temporarily interrupted by cutting. Examination of sections showed, in practically all cases, that the isolated stolon-piece had modified the budding process slightly, no matter how little time elapsed between isolation and appearance of the young bud. Specific cases are described.

In some cases the piece represents the very end of a stolon, in others

it lay at a little distance from the tip. The differences in the behavior of these two kinds were found to be negligible.

(1) A piece cut off at a little distance from the tip and  $1\frac{1}{2}$  mm. long when isolated, healed at each end within a few hours. It shrank slightly in both length and circumference within the first twenty-four hours, and formed a slight protrusion between the middle and one end within fifty hours. The piece was killed in Allen's modification of Bouin and cut into transverse sections of  $5\mu$ . The structure of this piece was found to be so similar to that of a normal budding stolon, at a corresponding stage, that special figures seem unnecessary. The facts are, however, stated and reference may be made to figs. 14, 15, 16. The ectoderm is completely healed over each end, but is columnar at only one end, the end nearer the bud. There is a septum but it is a little withdrawn from each end of the piece and, along its edges, from the dorsal and ventral ectoderm. Evidently the septum contracts as a result of the operation. At  $50\mu$  from the end the septum is a slightly thickened plate extending about half way across the haemocoel, with nuclei arranged alternately on the two sides of the plate. For the next  $80\mu$  the septum continues as a thin plate, showing in section a single row of nuclei. Following on this region the septum becomes thicker near one edge, showing in section a double row of nuclei, and forty-five micra farther on a slit-like cavity appears in this thicker portion separating two lamellae. The cavity grows in size as it is followed toward the other end of the piece, reaching a height of  $92\mu$  and a width of  $24\mu$ . Its entire length is  $125\mu$ . At no point does the cavity extend through the entire septum, i.e., a slender remnant of the original simple plate remains ventral to the vesicle as in normal budding. Throughout the extent of the vesicle lymphocytes and mesenchymal cells can be seen attaching themselves to its wall. Cells in the wall of the vesicle can be seen in mitotic division. The most obvious free blood cells of the haemocoel are of the highly specialized varieties, e.g., green cells, morula cells, and granular amoebocytes. These cells throughout the piece of stolon are found clumped and have an abnormal appearance as if beginning to disintegrate. Possibly they may be furnishing food for the growing bud. Mixed in with the abnormal cells can be seen the common type of tunic cell (compartmental amoeboid) but these usually appear normal. Practically all of the lymphocytes and mesenchymal cells have disappeared from the haemocoel; presumably they have been added to the septum to aid in blastogenesis. The few lymphocytes and mesenchymal cells which remain free in the haemocoel have the normal appearance.



*Thus in this piece the septum aided by lymphocytes and mesenchymal cells, as in normal budding but in greater degree, has begun to form an endoblastic vesicle.* Mitotic figures show that the wall of the vesicle is undergoing intrinsic growth. A noticeable difference from normal budding concerns the specialized haemocoelic cells. The beginning disintegration of these cells is correlated with the fact that the piece of stolon is cut off from the blood stream of the parent. Certainly the isolated piece of stolon encounters the problem of nutrition, and it would seem that this is solved by sacrificing the more specialized haemocoelic cells. At any rate the observations point in this direction.

(2) In another instance the abscised piece, 1.5 mm. in length, was taken well back of the stolon tip. In four hours after isolation, healing of the ends had taken place and they were withdrawn slightly within the tunic. In twenty-four hours a slight swelling appeared on the side of the piece about one-half millimeter from one end. The piece was immediately killed in Bouin without acetic acid and serial sections of  $5\mu$  made. The ectoderm cells at each end are found to be cuboidal. Covering each end is a thin layer of new tunic. Within the haemocoele, at the end nearer the swelling, there is no sign of a septum for  $30\mu$  when it appears as a plate thickened near one edge and contracted away from the stolon wall. The thickened portion shows at this level only a few nuclei and the cytoplasm no cell boundaries. In the thinner portion of the septum at this point there are no nuclei. Plainly the septum in this region has undergone contraction. This contraction of the septum away from the ectoderm is found to have occurred throughout the piece. It is greater at the ends than along the dorsal and ventral edges, and is especially great at the end farthest from the above mentioned swelling. For  $145\mu$  from this end the septum exists as a thin plate which occasionally shows a slight break and several irregularly thickened spots evidently formed by the addition of lymphocytes or mesenchymal cells from the haemocoele. The septum now becomes irregularly thickened near one edge (fig. 26). In this region there are some indications of amitotic cell division. Twenty micra farther on the enlarged portion of the septum acquires a fairly regular contour, triangular in the section and a good sized rounded cavity (fig. 27a). In the same region there can be seen in the haemocoele, beside the septum, a plate of cells similar to the septal cells in appearance and staining reaction. These are evidently mesenchymal cells or lymphocytes which have not yet added themselves to the blastogenetic mass (fig. 27b). The vesicular condition of the dorsal part of the septum continues for  $225\mu$ , beyond which

point the septum again becomes a single plate of attenuated cells with an occasional lymphocyte clinging to its sides. This condition persists through a stretch of  $420\mu$  and the septum then passes into a large terminal endoblastic vesicle which comes to an end  $200\mu$  from the actual end of the stolon (fig. 28).

This stolon-piece seems to be forming two endoblastic vesicles, one lateral, the other terminal; only the laterally placed vesicle could be recognized externally. One or the other or indeed both of these vesicles may have been present in an incipient stage when the piece of stolon was isolated, but their close proximity makes it very unlikely that both were forming at that time. Of course the terminal vesicle of the stolon-piece, if it did exist in the uninjured stolon, could only have existed as a lateral vesicle. The fact that the lateral vesicle is part of an externally perceptible bud may be taken to indicate that it is the older of the two. And the proximity of the terminal vesicle to this certainly suggests that the terminal vesicle is a new formation and not a mere modification of a lateral vesicle in existence before the operation.

(3) In a case very similar to the one just described, the abscised stolon-piece was only slightly over one mm. in length. This piece elongated very slightly and a lateral bud made its appearance. Sections reveal the presence of a very large lateral endoblastic vesicle in the recognizable budding area, and a smaller sub-terminal vesicle near one end of the piece. Within the cavity of the larger vesicle there are some free blood cells thus indicating that the vesicle may have been open to the haemocoel at some time. Gaps in the wall of the endoblastic vesicle sometimes occur in the natural budding process, and also free blood cells in the cavity of the vesicle. Evidently, then, free blood cells may enter the cavity from the haemocoel. Such gaps are evidently closed later on, either by intrinsic growth or by mesenchymal cells or lymphocytes. The free blood cells in the cavity of the vesicle are presumably absorbed. It may be noted in passing that the distinction between mesenchymal cells and lymphocytes is a formal one. Moreover, the similarity of these elements to the constituents of the septum as well as their fusion with the septum in regions of blastogenesis indicates that they all constitute a simple undifferentiated formative material the elements of which may assume different shapes and connections according to their temporary location, a point of view which is quite in accord with that of Brien and Brien-Gavage (2, 4). No definite organogenesis has begun in either of the two bud primordia of this piece, although the larger of the two is of a greater size than in the normal buds

at the time when organogenesis usually begins. The large vesicle does have its right side thickened preparatory to the formation of the endo-style. There are perhaps several reasons for the belated organogenesis of the larger vesicle, chief among which may be the lack of formative material which has perhaps been used up in the formation of the second and younger vesicle.

(4) Another stolon-piece, which was 1.3 mm. long when isolated, elongated slightly and budded in apparently normal fashion in about fifty hours. As the bud became noticeable the stolon contracted slightly in length and circumference. Examination of serial sections shows the following points. The ectoderm is stretched thin over each end of the haemocoel, and there is a delicate new tunic layer also covering each end. There is a well-formed, almost spherical endoblastic vesicle slightly nearer one end of the piece. No organogenesis has begun, but the entire wall of the vesicle is thickened as if in preparation for the process of rotation of the right wall to a ventral position (p. 199). Mitoses are numerous in the vesicle wall, and there are still irregular patches of lymphocytes and mesenchymal cells clinging to the wall where evidently they have recently migrated but have not yet been drawn into the wall itself (fig. 29, showing a part of the wall). The bud lies nearer one end, as said. In this part of the stolon the septum is still present in its usual plate-like form, but in the longer part of the stolon on the other side of the bud no septum is present. Here then it has evidently been entirely drawn into the endoblastic vesicle. Some of the more specialized blood cells of the haemocoel can be found clinging to the wall of the endoblastic vesicle in various places, but it is highly improbable that they are ever incorporated in the wall itself since no cytoplasmic connections are to be seen. The specialized cell most commonly attached to the vesicle wall is the familiar tunic cell, i.e., compartmental amoeboid. It is conceivable that they are concerned in the nutrition of the growing vesicle. At any rate such cells are apparently concerned in some measure with the general nutritive phenomena going on in the piece, for they are sometimes found free in the haemocoel with bits of other cells or entire cells in their cytoplasm (fig. 12a, b). This is evidently phagocytosis. Near one end of the haemocoel the compartmental amoeboid cells have formed a clump in which may be distinguished a few green cells apparently in the process of degeneration. Other small groups of blood cells show signs of degeneration, but there are many blood cells present that are perfectly normal in appearance.

(5) A stolon-piece 1.7 mm. long was cut out four or five millimeters

back of the stolon tip. Within the first two hours after isolation the piece shrank within the tunic and apparently healed at each end. Within the next twenty-four hours it elongated slightly. Within forty-eight hours a bud-like projection appeared on one side of the piece between the middle and one end. As the bud grew the stolon shrank. At the end of fifty-seven hours the piece was preserved in Allen's modification of Bouin and serial sections made. The ectoderm, as is usual, is columnar at each end. At the level of the bud-like projection there is a large endoblastic vesicle with a smooth regular wall thicker dorsally than elsewhere, and evidently close to the period of organogenesis. To it ventrally is attached a plate-like remnant of the septum. All this as in normal budding. The vesicle is prolonged toward one end of the piece into a septum, thin and plate-like ventrally, dorsally irregularly thickened and excavated by a cavity continuous with that of the main vesicle (fig. 30). This thickened part of the septum bears irregular projections and to it are attached strings of cells all evidently representing fusions of mesenchymal elements. Still nearer the end the septum ends much as in the normal, i.e., as a vertical plate thickest at its tip. On the other side of the bud there is no septum in the remnant of the stolon. Here then it must have been entirely absorbed in the endoblastic vesicle. In this part of the stolon too there are very few mesenchymal elements, apparently nearly all having been withdrawn into the blastogenetic center. The differences between the development of this piece and normal budding may be summed up: (1) The septum is absent on one side of the endoblastic vesicle, having been apparently drawn bodily into the latter. (2) The share taken by mesenchymal elements in the formation of the endoblastic vesicle is here (fig. 30) conspicuous. *It is clear then that the endoblastic vesicle is here developing less by growth than in the normal, and more by the direct utilization of material already present. And this statement applies to all the cases studied.*

(6) A piece 1.9 mm. long was cut from the distal end of an unattached stolon which exhibited no buds for a distance of 6-8 mm. Within the first twelve hours after isolation the piece grew one-half millimeter in length but decreased in width proportionally. The cut end, which apparently had healed completely, projected about one-fourth millimeter beyond the old tunic and was covered by a delicate new tunic layer. Within twenty-four hours after isolation a swelling appeared between the middle and the cut end of the piece, which was then killed in Allen's modification of Bouin and sectioned at  $5\mu$ .

In this piece there is a large endoblastic vesicle, close to organogenesis,

if indeed organogenesis has not already begun. And this vesicle, as in the preceding case, is prolonged on one side into a plate-like septum (about  $470\mu$  long) which, going toward the tip of the stolon, passes into a loose mass of rounded cells interconnected by cytoplasmic strands. This loose mass is quite long ( $240\mu$ ). Evidently there is here a deviation from the normal, apparently produced again by an increase in the passage of mesenchymal cells into the septum. Even where the septum is plate-like and thin, as occurs generally in these pieces, mesenchymal cells, are found attached here and there to it or fused in the shape of small plates close to it. In the wall of the vesicle are found not only mitoses but figures which suggest the occurrence of amitosis (fig. 31). Some free blood cells occur in the vesicular cavity as recorded for other cases. At the other end of the vesicle it also passes into a plate-like septum which extends toward the cut end of the piece. But this part of the septum is exceedingly short, only about  $10\mu$  long. A point may be mentioned in respect to the haemocoelic cells. The most abundant is the typical tunic cell or compartment cell some of which in this piece, both in the haemocoele and in the tunic of the tip of the piece, show a peculiarity not observed in any of the numerous preparations made either from normal stolons or from other cut pieces, although prepared with the same technique (Allen's Bouin, haemalum, acid fuchsin). The cells in question show red drops or vacuoles, which are larger than and do not resemble the granules brought out with neutral red in the living cells. George (6), to be sure, finds that the stainable granules in the living cell may agglutinate to form larger bodies.

Very little, if any, organogenesis has definitely begun in this young bud. In one spot near the anterior end of the vesicle a small group of lymphocytes has aggregated. This may possibly represent the earliest primordium of the dorsal tube. The primordium of the endostyle is also, it may be, represented on the thickened right side of the vesicle. The piece still has plenty of mesenchymal elements in its haemocoele.

In these longer isolated pieces of stolon, then, the bud primordium arises in the same general way as in the normal stolon. The septum shrinks from the sides (ends also, here) of the stolon, its cells multiply by mitotic and possibly amitotic division, and there are added to this septal mass lymphocytes and mesenchymal cells of the haemocoele. The differences are as follows. While in the formation of the endoblastic vesicle of the normal bud intrinsic growth of the septum and addition to it of mesenchymal cells both play a part, the symmetry of the growing structure (figs. 14, 15, 16) indicates that intrinsic growth is the dominant

factor. This results in the formation of a more or less symmetrical thickening which is then excavated. In the case of the isolated pieces, as has been said (p. 207) it would seem that the vesicle develops less by intrinsic growth and more by the direct incorporation of the septal material at the sides of the young vesicle and of mesenchymal elements of the haemocoel. Moreover, the section pictures, such as figs. 26, 28, suggest that the vesicle is sometimes formed, not by the excavation of a solid mass, but by the arrangement of mesenchyme and septum around a space. If this really occurs it is not surprising to find so often cells within the endoblastic cavity (fig. 31). Explanation of why two bud primordia form occasionally in such short pieces is not easy. In the normal, freshly collected material buds usually occur on the stolon at intervals of 1.5–2 mm. Some of the pieces which were only slightly over 1 mm. in length presented two buds primordia as has been described. The stolons from which these pieces were cut were usually unattached and without buds for a distance of 6–8 mm. Consequently there may have been present in these long barren areas an extra amount of reserve formative material, i.e., lymphocytes and mesenchymal cells, which may have been stimulated to action by the cutting, or by their freedom from the dominance of more highly organized centers, i.e., ascidiozooids. The matter of the nutrition of the young bud primordia is also problematical, but the discussion of this question is deferred.

#### *Organogenesis in the longer pieces of stolon*

In their further development the longer isolated pieces of stolon vary. In some of them organogenesis seems to proceed quite as in the normal bud, but in others the process differs considerably from the normal. Material for this portion of the investigation is not as plentiful as that for the study of the bud primordium, and there are consequently some gaps in the account. Specific cases are described.

(1) A stolon-piece, originally 1.5 mm. long, on the second day after isolation exhibited a normal looking bud borne laterally on the somewhat reduced stolon. Transverse sections of this piece reveal a well developed young bud in the stage of formation of dorsal tube and pericardial primordia. The dorsal tube primordium is a loose aggregate of a few lymphocytes  $50\mu$  long and lying in contact with the dorsal wall of the endoblastic vesicle near the anterior end of the latter. There is no organization as yet of this irregular mass into a rod or a tube. The primordium is very like the corresponding stage in a normally formed bud. The pericardial primordium adheres to the upper half of the right

side of the vesicle. It is a mass of loosely connected lymphocytes and the entire primordium has a length of  $20\mu$ . The vesicle itself has not undergone any torsion, although, from mid-region to the posterior end, the entire right side (future endostylar region), the dorsal wall, and the upper half of the left side are thickened, presumably in preparation for rotation. Throughout the length of the vesicle the entire septum has become two-layered, the cavity of the vesicle extending ventrally between the two thin septal layers. Lefevre (13) has recorded this condition and thinks the two layers eventually come together again. This case then reveals no recognizable deviations from the normal.

(2) A case in which the bud is slightly older than the preceding may be described. The stolon-piece, which was 1.7 mm. long when isolated, first shrank then increased slightly in length and appeared to bud normally. The piece was preserved about thirty-six hours after isolation. Sections show a well-developed bud. The primordium of the dorsal tube is  $60\mu$  in length, or about one-third the length of the entire vesicle, but as yet has not acquired a cavity nor has the ganglion been differentiated. This is about the condition of the nervous primordium in a normal bud at this stage. Left and right peribranchial chambers are forming although neither appears normal in size or method of formation. The right chamber is smaller than one would expect and exists as a tubular sac lying alongside the vesicle and anteriorly opening into the vesicle not far from the dorsal limit of the latter. Evidently there is some minor difference here from the normal method of formation. The left chamber differs from the right and from the normal. It exists as a short sac at the side of the vesicle, ending blindly in front, opening into the vesicle posteriorly. *Apparently in the formation of these chambers there are some real though minor growth differences from the normal.* It is surprising to find no sign of a pericardial primordium in this piece, and this point may be counted as a deviation from the type in the matter of times of formation of primordia. Whatever deviations from the type are exhibited in this piece are minor ones of morphogenesis. The cellular appearance shows that the bud is healthy.

(3) In another case the isolated stolon-piece was 1.7 mm. long. It failed to elongate, and curved roughly after the fashion of the letter U. After three days there was a swelling at each end. Sections show a well-developed terminal bud in the larger of the two ends, no septum in the intermediate region, and a loose collection of cellular plates and a few lymphocytes in the smaller end. In the bud of the larger end the pharynx is in communication with the two well-formed peribranchial

chambers by means of four series of stigmata. The oral and atrial siphons are both in process of formation, but no communication with the outside has been established. The dorsal tube primordium is now tubular and in communication with the pharynx. The ganglion has appeared as a small mass on the upper surface of the tube. The intestine is of considerable size and length and has begun to differentiate into regions; however, it has not yet established connection with the cloaca. The gut wall exhibits numerous mitoses. On the right side of the posterior part of the pharynx the pericardium and heart extend for a distance of  $15\mu$ . Some of the granular amoebocytes and green cells of the haemocoel appear in a degenerate condition; otherwise the cellular constituents look normal. *In this piece then there is terminal budding, and budding in the normal stolon of Perophora is lateral, more precisely dorsal.* In *Clavelina lepadiformis* budding is normally terminal: Brien and Brien-Gavage (3). Perhaps, however, the difference from the normal exhibited by this piece is not so radical since it may be that the cut was made at the exact spot of an incipient bud. The fact remains that the piece does exhibit terminal budding and that in correlation with this morphogenetic activity at one end the entire septum disappears, doubtless used up in the formation of the bud.

(4) Finally, there may be recorded a case at an advanced stage of development in which the organogenesis seems perfectly normal. When isolated the stolon-piece was 1.5 mm. long and  $\frac{1}{2}$  mm. wide; it was not the end of a stolon. During the first twenty-four hours the cut ends healed completely and both extended beyond the old tunic covering; the length of the piece doubled in this length of time. At the end of the second twenty-four hours the length of the stolon-piece had doubled again and a bud was borne laterally about midway on the stolon. The bud was allowed to develop for another twenty-four hours before the piece was killed. Although relatively very large as compared to the stolon, the bud had not assumed visible activities. Since normally all buds become obliquely inclined to the stolon with apices pointing toward the end of the latter, the distal part of the stolon may be recognized even in an excised piece by the inclination of the bud. And so in this case the inclined bud served to show which was the proximal and which the distal end of the piece. The proximal end was unhealthy in appearance and practically devoid of free cells. The distal end had a few free cells in slow circulation. Sections show that the distal part of the stolon has a one-layered plate-like septum but its haemocoel is very poor in mesenchymal content. The part of the stolon on the other



side of the bud is  $120\mu$  long but the septum is absent from the terminal portion through a length of  $50\mu$ . The organs of the bud seem to be perfectly normal. Neither oral nor atrial siphon has broken through but in both cases the external invaginations of the ectoderm have met the pharyngeal and cloacal sacs. The dorsal hypophyseal tube opens into the anterior end of the pharynx and extends backward on the mid-dorsal surface of the latter for a distance of  $50\mu$ . A ganglionic mass lies dorsal to the tube for the greater part of its length. The peribranchial chambers are in communication with the pharynx by means of three tiers of branchial stigmata. A well developed heart is present for a distance of  $45\mu$  on the right ventral side of the pharynx. Its size and appearance are normal, and it probably was in action at the time the bud was killed as a slow circulation of cells was noted in the more healthy distal end of the stolon. Between stolon and bud the septum is just breaking its connection with the left peribranchial fold. In this particular case the haemocoel of both stolon and zooid is unusually poor in free lymphocytes and also in the specialized blood cells. *The remarkable feature exhibited by this piece is its great growth in length and this may well be linked up with its paucity in blood cells at the time of fixation. They have been apparently largely used up.*

Summing up it may be said that *these abscised pieces of stolon do exhibit in their development certain minor deviations from the type: a growth difference in the establishment of the peribranchial chambers; a difference in the relative times of formation of organ primordia; a difference in the location of the budding region, it being terminal in one case.*

#### *Early behavior of the shorter pieces*

Pieces of stolon 1 mm. or less in length, usually failed to elongate and began, at least, the process of direct transformation into an ascidian body. A few of these pieces increased somewhat in length, then shortened as the ascidian developed. Several stages in the process of development of these short pieces are individually described.

(1) A piece of stolon which was  $\frac{3}{4}$  mm. in length when abscised healed at each end, shrank slightly in length, but increased in general diameter within twenty-four hours of isolation. One end of the piece appeared swollen within twenty-four hours and the piece was immediately preserved in Bouin without acetic. Sections show that ectoderm is complete at each end of the piece but columnar only at one end, the smaller. The septum has withdrawn for a distance of  $100\mu$  from this end, then appears in its typical plate-like condition, formed of a single layer of

cells. This condition persists, as the sections are followed forwards, for about  $60\mu$  when the septum is joined by a curved plate of mesenchymal cells, the two making a U-shaped structure as seen in section. This mass soon passes into an irregular structure (fig. 32) made up of a complex of trabeculae and the septum. That the complex is made up of plates attached to one another and the septum is obvious, and the histological structure shows that the plates represent fused mesenchymal cells. In many of the spaces between the plates are specialized blood cells, e.g., green and morula cells, which have been trapped during the formation of the complex. This irregular formative mass extends for a distance of  $200\mu$  toward the larger end of the stolon. In the  $80\mu$  between its termination and the end of the stolon there are only scattered cellular plates, a few free mesenchymal cells, and the specialized blood cells of the haemocoel. The majority of the specialized blood cells of the whole piece are packed closely around the irregular complex which is evidently to be looked on as an incipient blastogenetic mass.

Here then the entire septum has shrunk away from the ends toward the middle of the piece drawing with it practically all of the formative cells, thus giving rise to an irregular mass which doubtless would have developed further had opportunity been given. The green cells and compartmental amoeboid cells are the most numerous of the specialized cells. The latter have formed clumps at many points in the haemocoel. Many of these latter cells are exhibiting phagocytosis, the phagocytosed cell in most cases being one of the uncommon tunic cells (compare figs. 5 and 12a, b). Small groups of specialized cells in the haemocoel seem to be in the early stages of degeneration.

(2) Another short piece of stolon upon abscission was accidentally cut into two pieces, each less than one-half millimeter in length. These two pieces were allowed to remain very near together in the same tunic covering. After two days they fused completely and on the third day the fusion-piece had a smooth spheroidal contour and a very healthy green appearance (fig. 33). Sections show that the ectoderm is complete around the entire piece. Inside the haemocoel the majority of cells look normal and healthy. What doubtless is a remnant of the original plate-like septum is present (fig. 34). This lies in the central region of the mass. Near it and in part attached to it are some free mesenchymal cells, aggregations of mesenchymal cells, and little plates and masses obviously representing fused mesenchymal cells. There can be little doubt that this whole complex was in process of producing an endoblastic vesicle and not by the method of forming a solid mass which later is

excavated as is the case in normal budding (compare figs. 14, 15, 16). Some phagocytosis is going on. Of the phagocytes themselves some resemble compartmental amoeboids and others signet-ring cells (fig. 12a, b, c).

(3) Another stolon-piece, which was about 1 mm. long when cut, shrank about one-fourth millimeter in the first three hours after abscission, and at the end of twenty-four hours was only one-half millimeter in length and about as great in diameter. Sections show a complete covering of ectoderm, columnar at each end. In the haemocoel there is no definite septum. Instead there is found an aggregation of cell plates and cells surrounding in an irregular way a space or spaces (fig. 35) as if the formation of an endoblastic vesicle had begun in an atypical fashion (compare figs. 26, 28).

(4) Another piece which was only one-half millimeter in length when abscised was torn and bruised considerably in the cutting and was almost detunicated. Ten hours after isolation this piece had a curved or C-shape, was enlarged at one end, and had regenerated a new tunic. It was then fixed. Sections show that the ectoderm is complete around the entire piece. There is a recognizable remnant of the plate-like septum and this is connected with a large sac (fig. 36) the structure of which makes it almost certain that it has been formed not by the thickening of the septum followed by the excavation of the thickened part, but rather by an arrangement of cell plates and remnants of the septum around a space. To specify details, this conclusion is indicated by the very large size of the cavity and the thinness of the walls. Moreover there are enough blood cells in the cavity to be conspicuous. And, finally, the whole structure has been formed within ten hours. Many cases of phagocytosis are seen in the haemocoel of this piece. The phagocytic cells are of both the signet-ring and compartmental amoeboid types. The phagocytosed masses appear to be either bits of morula cells or of the more uncommon type of tunic cell.

(5) One short piece completed the formation of an endoblastic vesicle on the second day of isolation. When abscised this piece measured one-half millimeter in length and not quite one-fourth millimeter in diameter. Its length remained the same, but its diameter seemed to increase slightly, due perhaps to absorption of water. Sections show that the ectoderm is hardly complete over one end, but is entirely healed over the other. The end which failed to heal was bruised in cutting. There is a large endoblastic vesicle,  $115\mu$  long,  $64\mu$  high,  $40\mu$  wide. The entire wall is thin, the nuclei forming a single layer and showing mitotic figures.

There is only a short remnant of the septum, and there are very few mesenchymal cells in the haemocoel. This piece is, perhaps, less convincing than the preceding, but the large size of the cavity and the thin wall of fairly uniform thickness make it on the whole improbable that the vesicle has been formed in the typical way, i.e., by the establishment of a solid mass which is subsequently excavated (compare figs. 14, 15, 16). Many of the specialized cells of the haemocoel are in a degenerating condition; this is particularly true of the green cells, morula cells, and granular amoebocytes. The degenerating cells are closely packed around the greater part of the outside of the endoblastic vesicle and it is possible that some of this material may have been absorbed by the vesicle. Many signet-ring cells have solid inclusions in their vacuoles. Some compartmental amoeboid cells also exhibit phagocytosis.

#### *Organogenesis in the shorter pieces*

After the establishment of the endoblastic vesicle early organogenesis seems to proceed much as in normal budding.

(1) A stolon-piece three-fourths millimeter long when cut may be described. Within three hours after isolation this piece had healed at each end, had shrunk slightly within the tunic, and there was a decided difference in size in the two ends of the piece. Within twenty-four hours after isolation a well-formed bud was borne very close to the distal end of the stolon, this end barely projecting beyond the bud; the diameter of the stolon was reduced to one-half its original size. Sections show a well-formed and very large endoblastic vesicle. A plate-like septum is present in the long part of the stolon and in the region of the bud a septal remnant extends ventrally from the endoblastic vesicle in the usual way. Close to the vesicle in their usual locations the primordia of the neural tube and pericardium are in process of formation. The dorsal tube primordium is as yet an unorganized mass, about  $55\mu$  long, irregular in shape in spots and again flattened, and not differing from the corresponding stage in the long pieces or in the normal bud. The pericardial primordium is about  $40\mu$  long and lies, as in the normal bud at this stage, high on the right side of the vesicle. This primordium is organized into the form of a flattened tube for about half of its length and thus is evidently developing as in the normal bud; as in the normal bud it is somewhat ahead of the dorsal tube primordium in organization. Both primordia are, in spots, closely applied to the wall of the endoblastic vesicle, but, as in normal budding, there is no definite

sign of cells passing from the wall of the vesicle into the primordia. In the haemocoelic cavities of both bud and stolon there are very few free mesenchymal cells or lymphocytes left, a fact which indicates that they were used in the establishment of the endoblastic vesicle and the organ primordia.

(2) In another case the stolon-piece, originally 1 mm. long, was allowed to develop four days before being killed. A bud of considerable size was formed; the stolon was reduced in length as the bud developed. Serial sections show a large pharynx from which the peribranchial sacs are being separated in the usual way, and there is an evagination, the esophagus, growing out from the left posterior region of the pharynx. The dorsal tube primordium is a compact, definitely outlined mass about  $50\mu$  long, in the usual position, offering no difference from the normal. In a normal bud of this stage of development the pericardial primordium is relatively large and has already become saccular. In the piece under study its only representative is a solid little mass of cells about  $5\mu$  long, lying however in the typical position of the primordium. The formation of the pericardial primordium is evidently greatly retarded in this piece. One may say that an attempt, so to speak, is being made on the part of the bud to develop its digestive and respiratory apparatus relatively faster than its circulatory system, whereas in normal development the primordium of the pericardium is one of the earliest to appear. A similar retardation in the development of the heart was described for one of the longer pieces of stolon.

(3) A final case may be described in which the piece of stolon was rather long, 1 mm., and in which the bud was allowed to develop into an ascidian body. Both ends healed within a few hours, and in the first twenty-four hours the piece showed a slight increase in length. A swelling appeared laterally near one end of the piece, and as the swelling increased the stolon shrank in length correspondingly. The bud was allowed to develop for thirty-six hours before being killed in Bouin. At this time the bud was  $112\mu$  high and  $144\mu$  wide; the total length of the stolon only  $345\mu$ ; the bud lateral and nearer one end of the stolon. Sections show that the septum has disappeared throughout the stolon except at one end where there is a little remnant,  $40\mu$  long. Very few mesenchymal cells are left. Evidently then the bulk of the septum and mesenchyme went into the formation of an endoblastic vesicle and the other internal primordia. The following organs are present and in fairly normal states and relations; pharynx, gut, peripharyngeal chambers, cloaca, nervous system, pericardium and heart. The gut has not

yet reached the cloacal wall; however it is already differentiated into three well-defined regions. Atrial and oral siphons have not completed their formation, yet both are indicated. Branchial stigmata are not yet formed, although the inner wall of the peribranchial chamber is in contact with the pharyngeal wall in many places.

#### DISCUSSION AND CONCLUSIONS

1. In the non-blastogenetic parts of the stolon of *Perophora viridis* the septum is a thin plate representing a single layer of cells. In the establishment of an endoblastic vesicle the septum takes the leading part. In this blastogenetic process it thickens in its dorsal region, largely, it seems clear, as a result of intrinsic growth, and secondarily by the addition of some free mesenchymal elements. This symmetrical dorsal thickening then becomes hollowed out to form a vesicle which may or may not locally involve the entire septum (figs. 15, 16).

This description of the septum and formation of the endoblastic vesicle agrees essentially with that given by Brien and Brien-Gavage (4) for *Perophora listeri*. It differs from that given by Kowalevsky (10), Ritter (15), and Lefevre (13), who describe the septum as primitively double and the endoblastic vesicle as arising by a growth process which is virtually an evagination.

2. In the case of the longer isolated pieces of stolon (1.1 to 1.9 mm. in length) the external appearance would indicate that the internal morphogenetic processes were very similar to those of normal budding. There are, however, differences of some interest. Section pictures show that the dorsal region of the septum thickens, but less by growth than by addition of a considerable amount of mesenchyme both in the form of cell plates and little cell masses. In this way an irregular blastogenetic mass is produced (fig. 30) into which a large part of the extra-blastogenetic portion of the septum is drawn (p. 207); however there is usually a recognizable remnant of the septum left in the stolon on one side of the vesicle. In these pieces, then, it seems that the formative materials (septum and mesenchyme) are making an adaptation in harmony with a decreased food supply. The intrinsic growth of a localized region of the septum being checked, the nearby formative material is directly drawn upon to a greater degree than in the normal.

3. The differences between morphogenesis in the very short isolated pieces of stolon (1 mm. and less) and in the normal bud are striking. The typical process of formation of an endoblastic vesicle mainly by local growth of the septum is distinctly departed from. In such a piece

in fact the septum may be difficult to recognize, for it may either be broken into small remnants not distinguishable from similar mesenchymal plates (fig. 35) or greatly obscured by the addition of plates and masses of such cells (fig. 34). The microscopic pictures are quite opposed to the idea that these formative elements unite to form a thickened mass which is later hollowed out to form a vesicle. No such mass is found. On the contrary pictures indicate that the plates of formative tissue (septum and mesenchyme) combine at first in a very irregular way to enclose a space or spaces (figs. 34, 35). By a process of rearrangement these irregular plates may form, it is thought, large vesicles with fairly uniform walls (fig. 36), such as are sometimes produced within a very short time after isolation (10 hrs. in one case) of the piece. Some of the section pictures of longer pieces (fig. 26) suggest that in them also the septal and mesenchymal constituents may arrange themselves around a space present from the beginning.

This interpretation of the microscopic pictures seems to be the only justifiable one. It is clear that the morphogenetic process in these little pieces, not only cut off from the parental food supply, but left with a minimum of formative material, undergoes a striking and what may be regarded as a qualitative change. The endoblastic vesicle, it would seem, is established in the quickest and easiest way by assembling together all of the available formative material into an arrangement which, though rough, has from the start the idea of a vesicle.

There are some slight differences between the process of formation of an endoblastic-vesicle, as outlined here, and that described by Brien (2) in the regeneration of experimentally isolated stolon tips of *Clavelina lepadiformis*. In these abscised stolon tips the formative elements are the same as in the *Perophora* stolon, namely, septum, mesenchymal cells, and lymphocytes. But the septum first contracts strongly (effect of the cutting), and to the contracted septum are added other formative elements, the whole making a solid formative mass which is subsequently hollowed out to form an endoblastic vesicle. Brien finds that in normal budding of *Clavelina* the septum breaks up into its constituent cells which later aggregate along with the other formative elements to form a solid blastogenetic mass which is then hollowed out to form the inner vesicle of the ascidiozoid. While the departures from the typical morphogeny exhibited by the isolated pieces of *Perophora* are much greater in the short than in the longer pieces, they are in them all of such a kind as to warrant looking on them as adaptive.

4. In the case of the longer pieces the development is one of mixed

growth and morphallaxis. In the case of very short pieces development is close to if not entirely pure morphallaxis. Wilson (21) records that occasionally such a little piece may entirely transform into an ascidian, the little remnants of the stolon persisting in such cases as have been here described being completely retracted into the body. In the parallel case of the transformation of pieces of *Clavelina stolon* Della Valle (5) found that a very small piece might be wholly transformed into an ascidian body, while in the case of a larger piece a part (terminal) becomes body and a part persists as stolon. Della Valle regards the morphogenetic processes in the latter case as closer to regenerative budding than to morphallaxis.

5. In the processes of organogenesis the majority of isolated pieces differ less from the normal buds than in the establishment of the endoblastic vesicle. The two most noticeable differences are concerned with the retardation of development of the heart primordium and with the smaller size and somewhat different method of formation of the peribranchial sacs. The former difference is probably correlated with the lack of sufficient formative material.

6. The formative material consists of septal cells, mesenchymal cells, and lymphocytes, and, as has been said (p 205) the distinction between these elements is a formal one. The significant fact is that they are all simple undifferentiated elements, very unlike the highly specialized and conspicuous green and morula cells which take no part in the formative processes.

Lefevre (13), while first to demonstrate conclusively that, in the ascidian bud, free cells of the blood aggregated to form organ primordia, nevertheless failed to identify these cells as any particular kind of blood cell.

Schaxel (16) states that it is the undifferentiated cells, to be found within the ectodermal and entodermal epithelia, and among the mesenchyme (blood cells) of the isolated branchial basket of *Clavelina lepadiformis* which are solely responsible for the regenerative process. Before the regeneration occurs, he believes that all specialized tissues disintegrate leaving only undifferentiated cells of the three layers. These regenerative cells, he finds, retain, as they proliferate, their original relative positions, i.e., ectoderm outside, entoderm inside and mesenchyme between the two. Thus a three-layered sac is produced which is, as he says (loc. cit. 139), quite like the stolon of a budding region.

Spek (18), on the contrary, thinks that the totipotent regenerative elements in the isolated stolon tips (winter buds) of *Clavelina lepadiformis*



*mis* are certain special amoeboid drop-cells. He also assigns a nutritive rôle to these cells under certain conditions. He finds that the drop-cells are manufactured in the wall of the mid-gut.

Brien and Brien-Gavage (2, 3, 4) find that the formative regenerative tissue in both *Perophora listeri* and *Clavelina lepadiformis* consists solely of the unspecialized cells, that is, the septum, mesenchyme, and lymphocytes, and, as said, they make no great distinction between these elements. The results of this investigation are in accord with those of Brien and Brien-Gavage in this matter. Brien (2) has found Spek's drop-cells in *Clavelina* and has analysed their inclusions as glycoproteins. He thinks that these cells may play an important trophic rôle in the budding or regenerating stolon but are not formative.

Wiegand (20), in his experiments on *Clavelina*, in which the upper part of the body is regenerated from the visceral sac, finds that in the regenerating region Spek's cells are absent, although abundant elsewhere. They cannot, therefore, be looked on as the regenerative elements. Wiegand, again, was unable to find in the visceral sac cells corresponding to Schaxel's indifferent reserve cells which the latter regards as the regenerative elements. As to the actual origin of the regenerative cells in this process, Wiegand came to no conclusion.

7. Cells exhibiting phagocytosis appear frequently in the abscised pieces, rarely in the normal stolon. Brien (2) finds in two of the *Clavelinidae* both in normal budding and in regeneration that certain large phagocytic cells are a constant and abundant element in the haemocoel of the stolon. He thinks these phagocytic cells are preying upon the more specialized cells of the stolon which have begun histolysis.

#### ABSTRACT

In normal budding of the social ascidian, *Perophora viridis*, the inner vesicle of the young bud is formed chiefly by local intrinsic growth of the stolonial septum. The septum itself is not a two-layered structure, that is, virtually a closed sac, but a simple partition representing a single layer of cells. To the growing septum, mesenchymal itself in origin, are added a few of the free unspecialized mesenchymal cells of the stolonial cavity, the two combining to form a symmetrical thickening in the dorsal region of the septum. This mass is excavated to form the cavity of the inner vesicle of the bud.

Isolated pieces of stolon when transforming into small ascidiozooids depart from the normal morphogenetic method employed in budding, the departure being greater in short (1 mm. or less) pieces than in

longer (1-2 mm.) pieces. In longer pieces the blastogenetic region of the septum may grow slightly but it depends largely on the incorporation of extra-blastogenetic parts of itself and on the addition of free unspecialized mesenchymal cells in attaining the vesicular state. In the short pieces the septum as such is scarcely recognizable. The section pictures indicate that remnants of it and mesenchymal plates and masses arrange themselves around a space to form the wall of a vesicle. The establishment of an endoblastic vesicle out of formative material already present instead of its establishment by a process of true growth, i.e., formation of new tissue, is a process of morphallaxis as contrasted with ordinary regeneration.

The formative elements in both budding and morphallaxis are the unspecialized undifferentiated cells of septum and blood stream.

CHAPEL HILL, N. C.

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## EXPLANATION OF PLATES 15-17

(All figures, unless otherwise stated, were drawn under oil immersion at a magnification of 1490 and with the aid of a camera. The original figures have been reduced by two-fifths.)

## Abbreviations Used in Figures

ectd .....	ectoderm	n. t. pr.....	neural tube primordium
end. ves }	endoblastic vesicle	pc. pr.....	pericardial primordium
end. v. }		sep.....	septum
fus. pc.....	fusion piece	sep. rem. }	septal remnant
haem.....	haemocoel	sep. r. }	
mes.....	mesenchyme	si.....	sinus
mes. c.....	mesenchymal cell	tun.....	tunic
mes. pl.....	mesenchymal plate	tun. c.....	tunic cell
mes. proj.....	mesenchymal projection	vac.....	vacuole
mes. m.....	mesenchymal mass		

## PLATE 15

- Fig. 1. Photograph of a colony of *Perophora viridis* grown on a slide in the laboratory. About natural size.
- Fig. 2. Cross section of a stolon in a non-budding region, 750  $\times$ .
- Fig. 3. Part of the end of a living stolon stained in weak neutral red showing the more common type of tunic cells with granules, and the thick ectoderm.
- Fig. 4. Fixed and stained tunic cells of the more common type, (a) from the haemocoel, (b) and (c) from the tunic.
- Fig. 5. Tunic cells of the more uncommon type, fixed and stained.
- Fig. 6. Green cells, (a), living; (b) and (c), fixed.
- Fig. 7. Morula Cells, (a), living; (b) and (c), fixed.
- Fig. 8. Signet-ring cell, fixed.
- Fig. 9. Granular amoebocyte, fixed.
- Fig. 10. Lymphocytes in varying aspects, fixed.
- Fig. 11. Mesenchymal cells, fixed.
- Fig. 12. Phagocytes from regenerating stolon pieces, fixed.
- Fig. 13. Transverse section of distal end of septum in a normal stolon.
- Fig. 14. Transverse section of thickened or blastogenetic septum just proximal to the tip.
- Fig. 15. The same septum from a transverse section of a still more proximal region of the stolon. A cavity has been hollowed out in the thickened dorsal portion.
- Fig. 16. The same septum, from a still more proximal transverse section, at the place of fullest extent of the endoblastic vesicle of the bud. The vesicle does not involve the entire septum.

## PLATE 16

- Fig. 17. From a transverse section of stolon; (a) an endoblastic vesicle which has involved the entire septum; (b) a plate of mesenchymal or formative cells in the haemocoel beside the endoblastic vesicle.
- Fig. 18. Septum as seen in transverse section, showing a single row of cells (nuclei), with mesenchymal plate projecting from the side.
- Fig. 19. Transverse section of thickened or blastogenetic septum exhibiting mitotic division and showing mesenchymal cells being added to it.
- Fig. 20. Semi-diagrammatic transverse section through a stolon and young bud showing the endoblastic vesicle and the earliest organ primordia: neural tube and pericardium (due to reversal under the microscope the left side of the figure, with the pericardial primordium, represents the right side of the bud)  $\times 170$ .
- Fig. 21. Dorsal portion of the picture shown in figure 20, enlarged to show the neural tube primordium.
- Fig. 22. Portion of the left side of fig. 20 enlarged to show the pericardial primordium.
- Fig. 23. From a transverse section, showing two blood sinuses in the ventral region of a young bud.
- Fig. 24, a, b. The two divisions of the stalk which connects bud and stolon, as seen in transverse section.

- Fig. 25. The undivided part of the stalk, close to the stolon, as seen in transverse section.
- Fig. 26. From a transverse section of one of the longer abscised pieces of stolon, showing the septum in early blastogenetic activity. Note its irregularly thickened dorsal region and the mesenchymal cells being added to it.
- Fig. 27. From a section of the same series as fig. 26: (a) endoblastic vesicle, an irregular asymmetric mass; (b) a plate of mesenchymal cells close beside the young vesicle in the haemocoel.
- Fig. 28. From a section of same series showing large terminal endoblastic vesicle.

## PLATE 17

- Fig. 29. From a transverse section of one of the longer abscised pieces. Part of the wall of a large endoblastic vesicle is shown. Attached to its outer surface are two little masses of mesenchyme and a remnant of the septum.
- Fig. 30. From a transverse section of one of the longer pieces of stolon, showing an early stage in the formation of an endoblastic vesicle. To the irregular, excavated mass representing the vesicle are attached a considerable remnant of the septum and an equally large mesenchymal plate, *mes. proj.*
- Fig. 31. Endoblastic vesicle, inclosing some haemocoelic cells, as seen in transverse section of one of the longer pieces of stolon. Mitosis is in progress in the cells of the vesicle wall.
- Fig. 32. Very irregularly formed blastogenetic mass. From a transverse section of a short piece of stolon.
- Fig. 33. Surface view of a regenerative mass, *fus. pc.*, resulting from the fusion of two very short abscised pieces of stolon, still in the old tunic, *tun.*  $\times 50$ .
- Fig. 34. From a transverse section through the fusion-piece shown in fig. 33. Septal remnants, mesenchymal plates and masses fusing around a cavity.
- Fig. 35. From a transverse section of a short abscised piece of stolon, showing irregular formative mass made by the fusion of septal remnants and mesenchymal cells.
- Fig. 36. Large irregularly shaped endoblastic vesicle, as seen in transverse section of one of the shorter pieces of stolon.

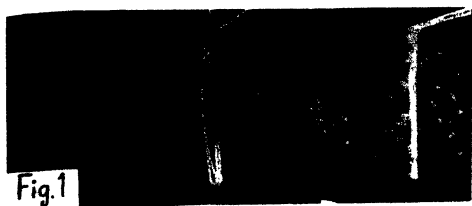


Fig. 1

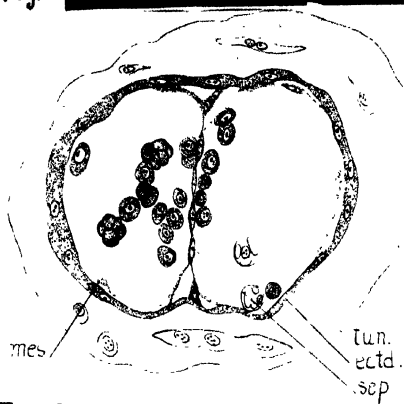


Fig. 2

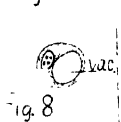


Fig. 8



Fig. 4

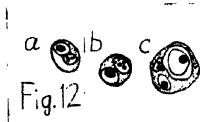


Fig. 12



Fig. 14



Fig. 13

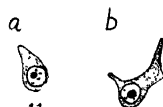


Fig. 11

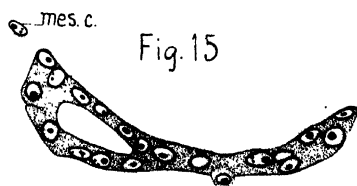


Fig. 15

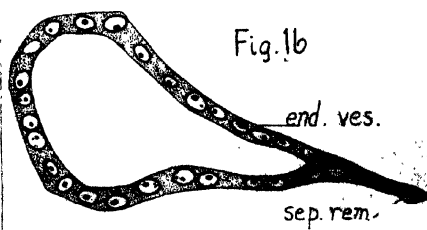


Fig. 16



Fig. 3

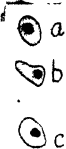


Fig. 5

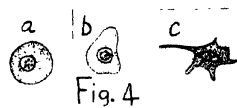


Fig. 4

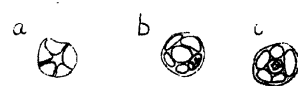


Fig. 6

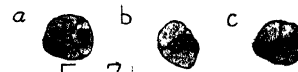


Fig. 7



Fig. 10



PLATE 16

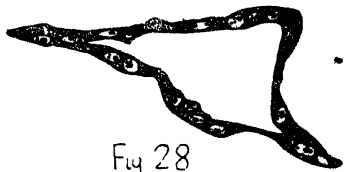
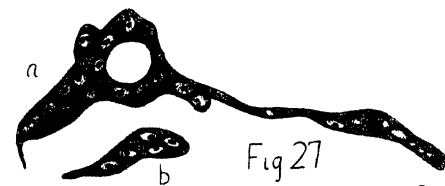
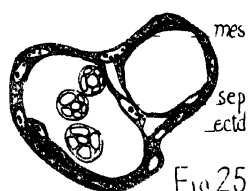
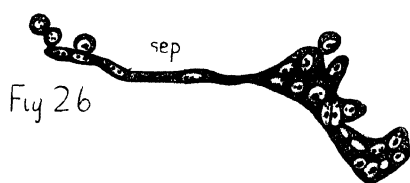
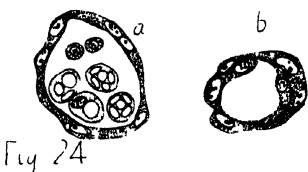
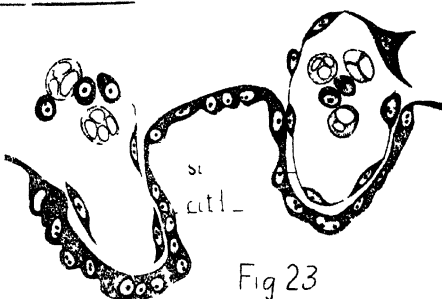
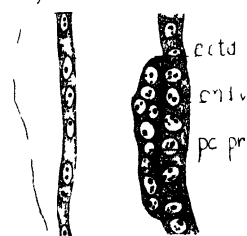
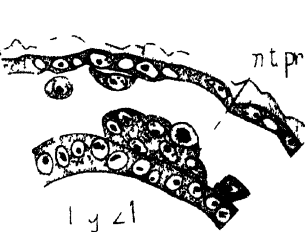
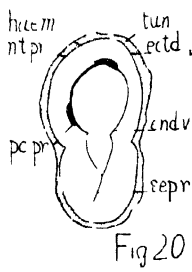
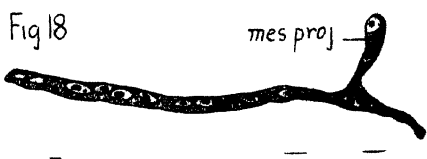
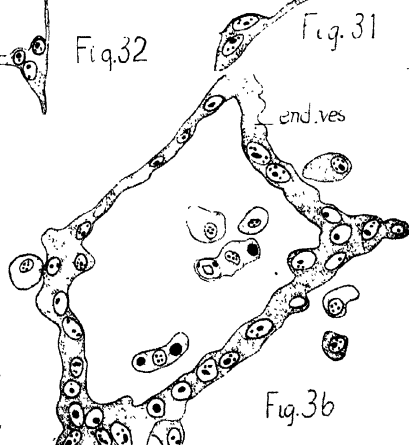
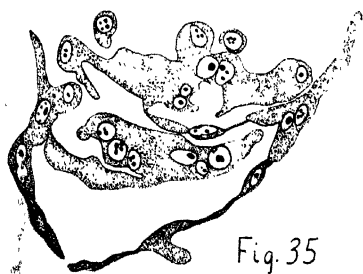
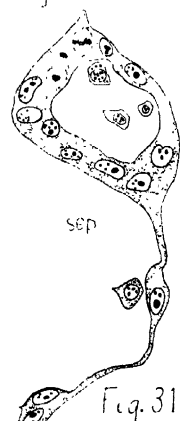
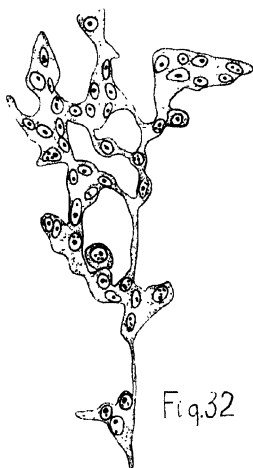
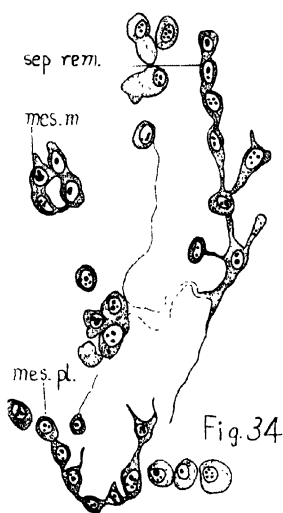
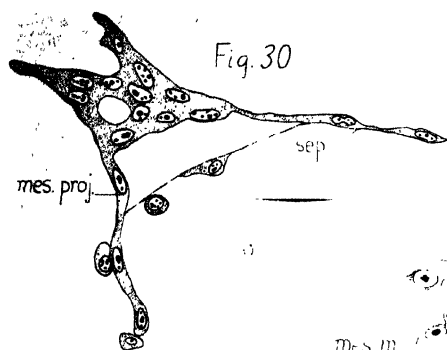






PLATE 17





## THE OLD FIELD PRISERE: AN ECOLOGICAL STUDY

By W. M. CRAFTON AND B. W. WELLS

PLATES 18-20 AND 1 TEXT-FIGURE

### INTRODUCTION

In the eastern mesic forest region the old field vegetation is an ecological unit strikingly distinct from that of the climatic climax vegetation. The primary stages of the developmental vegetation, the prisere, consist of an herbaceous flora entirely. This is followed by the tree flora which constitutes the dominant natural vegetation of the eastern United States.

The herbaceous flora, composed of grasses and weeds, ranges throughout old fields and waste places in a more or less regular series. In the southern upland bare areas the most frequent pioneer is the crab grass (*Syntherisma sanguinale*). It is particularly abundant in recently cultivated fields. It enters during cultivation and maintains dominance rarely after the second year. The general seeding and growth of invaders result in the exclusion of this annual. These invaders—tall weeds (*Eupatorium*, *Aster*, *Solidago*, and others)—play the dominant rôle for a time. A few years later the old field vegetation changes again and the third phase is initiated. This time broom-sedge (*Andropogon* spp.) invades—reaches its extreme expression—then passes out with the ecesis of pines (*Pinus taeda*, *P. echinata*, and *P. virginiana*).

Where the tree vegetation is open in stand in the old pine fields, broom-sedge is well represented. On the other hand, where the pines form dense consocieties by mass invasion this grass soon becomes only a relict of the final prisere stage.

The rate of establishment of the elements of the prisere are profoundly influenced by the edaphic and aerial conditions. Further, the sequence, in part at least, bears a direct relation to the ecesis of the final prisere element. This study deals primarily with the habitat in relation to the distribution of the vegetation. With this approach, certain conclusions can be drawn to account for the definite stages in the prisere.

It is not intended to discuss or even mention all of the plants in the old fields. The species under observation proved of interest because of the

aspects they present, importance in the habitat analyses, and, of course, their direct relation to the prisere.

#### METHOD

The studies of this problem were confined to the fields about Raleigh, North Carolina. For the measure of plant invasion and competition meter quadrats were marked off in several communities of different soil habitat. The vegetation in the quadrats was recorded late in 1930 and early spring, summer, and fall of 1931. Bare areas were made in the spring of 1931 by spading up meter square plots. Records of plant growth were obtained as in the other quadrat areas. In many of the quadrats, 20 cm. intra-quadrats were marked off. This permitted closer checking of the effect of competition. Further, a rather detailed study of a terraced slope was made.

The water supplying power of the soil was obtained only for comparative purposes. In certain striking situations texture analyses of the soil were made. Further, relative light values were obtained. All of these data were gathered from the various representative communities.

Information in regard to seedling growth and response to drought proved significant in the interpretation of the stages of the prisere development. This information was obtained from studies in both the green house and the field.

Close observation of the old field vegetation coupled with the data gathered from the above sources comprised the mode of attack on this problem.

#### THE VEGETATION

Like all primary vegetation, that of the old fields is somewhat xeric in nature. The upland areas tend to display a great many species adapted to the xeric habitats; the lowlands contain those species adapted to the more mesic conditions.

Usually the vegetation in either situation is so variegated that the casual observer sees nothing but a heterogenous mass of grasses and weeds. However, close and frequent observations will disclose a rather graded series of species in the revegetation of old fields. The appearance of this series is related to the nature of the soil and the species involved.

#### *Successional stages of the old field associates*

*The pioneer consocieties.* It is a common observation that cultivated fields become covered with crab grass. Bermuda grass (*Cynodon*

*Dactylon*) is less common as a dominant. Here and there occasionally appears *Paspalum* sp., but its occurrence is rare. In local moist areas cockle bur (*Xanthium commune*) and smart weed (*Polygonum* spp.) often become subdominants or even dominants. However, in upland fields they are always minor elements. In eroded fields where the subsoil is exposed, the pioneer stage is represented by ragweed (*Ambrosia artemisiifolia*), button weed (*Diodia teres*), and poverty grass (*Aristida* sp.). These exposed clay areas and the *Cynodon* consociates have the effect of holding up the usual rapid development of the prisere.

The annual crab grass often retains its dominance for a second growing season. At this time horse weed (*Leptilon canadense*) is found in addition to the minor plants of the previous year (pl. 18, fig. 1). This is the

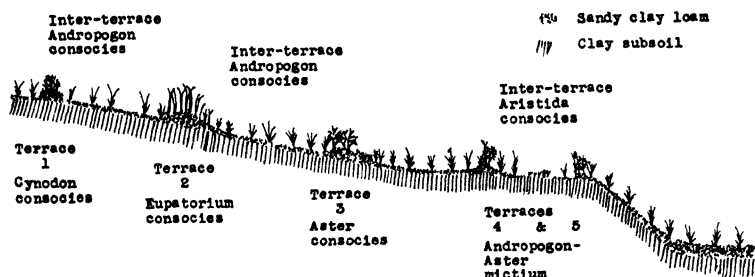


FIG. 1

most conspicuous plant of the associates because of its great height. Aster or some other tall weed may become well represented at this time, or at least the year following. Orchards, cultivated three or four times during the growing season, display crab grass and scattered aster. Foxtail (*Chaetochloa* spp.) is often present. Fields from which small grain crops have been harvested in the early summer, are given over to these grasses. Other fields which have reached the weed stage prior to planting to small grain sometimes skip the crab grass stage. Such fields are usually disked. After harvest the weeds become dominant again. Crab grass and foxtail may be found scattered throughout these areas representing remnants of the true pioneer stage. In sections of the upper piedmont it is a fairly common practice to prepare the fields in the summer for fall sowing. The plowing is usually done in July. With a few rains the fields become covered with crab grass.

Other plants in recently cultivated areas are mostly annuals. Occa-

sionally a few perennial weeds become established, but they are never abundant. Even the regular minor elements are more or less widely scattered.

The most striking aspects in this stage are contributed by toad-flax (*Linaria canadensis*) and tall sheep sorrel (*Rumex stricta*). In the sandy regions of the state they present beautiful vernal aspects of lavender and crimson. Fields cultivated the previous year are often covered by either or both of these plants. However, in the piedmont they are less common. Ox-eye daisy (*Chrysanthemum Leucanthemum*) and ragwort (*Senecio Smallii*) appear in the pioneer stage only in Bermuda grass areas which have been established for several years (fig. 2). Further, these species are much more common in the piedmont and mountain areas.

#### LIST OF THE CHARACTERISTIC PLANTS OF THE PIONEER CONSOCIES

##### *Dominants*

Ambrosia artemisiifolia	Paspalum sp.
Aristida sp.	Syntherisma sanguinale
Cynodon Dactylon	

##### *Subdominants*

Chaetochloa glauca	Leptilon canadense
Chaetochloa viridis	

##### *Minor plants*

Allium sp.	Erigeron spp.
Amaranthus retroflexus	Eragrostis sp.
Amaranthus spinosus	Hypericum gentianoides
Antennaria plantaginifolia	Linaria canadensis
Bidens spp.	Oenothera lacinata
Brassica sp.	Oxalis stricta
Chenopodium album	Panicum capillare
Chenopodium Botrys	Panicum virgatum
Chrysanthemum Leucanthemum	Rubus sp.
Convolvulus arvensis	Scleranthus annuus
Coreopsis sp.	Sida spinosa
Datura sp.	Solanum carolinense
Diodia teres	Xanthium commune

*The tall weed consocieties.* This is the intermediate stage, composed mainly of aster (*Aster* spp.), golden rod (*Solidago* spp.), dog fennel (*Eupatorium* spp.), sneeze weed (*Helenium tenuifolium*), and Carolina golden rod (*Euthamia caroliniana*). The last three are often dominant in neglected pastures. With grazing these weeds retain prominence

for years. However, when this factor is withdrawn, broom-sedge assumes dominance. This grass, though long seeded, is apparently held in check by grazing.

Frequently one observes succession within a weed consocieties. In the coarser soils dog fennel may be followed by *Aster*. However, the former may not be entirely eliminated until the final stage is initiated.

In the open weed communities the dominants of the pioneer stage strongly resist the invaders. These grasses are best represented in short weed consocieties, that is, communities of Carolina golden rod and sneeze weed. On the other hand, where the plant cover is heavy there is only occasional evidence of the retiring species.

A longer time is required for the suppression of Bermuda grass than any other of the pioneers. In many cases it is represented throughout this stage as an underplant community. It is entirely eliminated only in dense stands of weeds. Otherwise, it may persist long after broom-sedge has taken possession of contiguous areas.

Of all these weeds, the dog fennels (*E. hyssopifolium* and *E. capillifolium*) are probably the most important ones which extend far into the coastal plain. *Solidago odora* appears in the lower sand regions where clay is near the surface. *Aster ericoides*, most common in the piedmont, also disappears in the sandy regions of the state.

In open weed communities, daisy fleabane (*Erigeron* spp.), rabbit foot clover (*Trifolium arvense*), and hop clover (*T. agrarium*) represent the spring aspects. However, where the weed stand is dense these plants are apt to be eliminated.

#### LIST OF THE CHARACTERISTIC PLANTS OF THE TALL WEED CONSOCIETIES

##### *Dominants*

<i>Aster ericoides</i>	<i>Solidago odora</i>
<i>Aster Tradescanti</i>	<i>Solidago rugosa</i>
<i>Eupatorium capillifolium</i>	

##### *Subdominants*

<i>Achillea millefolium</i>	<i>Euthamia caroliniana</i>
<i>Eupatorium hyssopifolium</i>	<i>Helenium tenuifolium</i>
<i>Eupatorium</i> sp.	

##### *Minor plants*

<i>Antennaria plantaginifolia</i>	<i>Lespedeza striata</i>
<i>Bidens</i> spp.	<i>Lespedeza procumbens</i>
<i>Daucus carota</i>	<i>Trifolium agrarium</i>
<i>Desmodium acuminata</i>	<i>Trifolium arvense</i>
<i>Desmodium</i> sp.	<i>Rubus</i> spp.
<i>Erigeron</i> spp.	<i>Senecio Smallii</i>
<i>Lactuca canadensis</i>	<i>Solanum carolinense</i>



*The broom-sedge consocieties.* This final consocieties of the prisere may be found in various stages of expression. In different fields it is often observed sharing an area with tall weeds, short weeds, pines, or almost by itself (figs. 3, 4, and 5). With tall weeds it represents the part of an invader. Where the short weeds are found a more advanced stage is represented. The extreme expression of dominance is reached when even the short weeds are excluded. This latter situation, however, is of rare occurrence.

A few differences in the distribution of the tall weeds in the piedmont and coastal plain have been pointed out. The broom-sedge stage in these two sections of the state is represented mainly by *Andropogon virginicus*, *A. Elliottii*, and *A. argyraeus*. However, in the coastal plain *A. glaucopsis* becomes prominent in addition to *A. virginicus*.

In connection with this stage it is well to mention the contributors to the spring aspects. Fleabane (*Erigeron* spp.) and ragwort (*Senecio Smallii*) appear locally in broom-sedge areas. The appearance of rabbit foot clover and hop clover is more or less limited.

#### LIST OF CHARACTERISTIC PLANTS OF THE BROOM-SEGE CONSOCIETIES

##### *Dominants*

*Andropogon Elliottii*

*Andropogon virginicus*

##### *Subdominants*

*Andropogon glaucopsis*

*Euthamia caroliniana*

*Eupatorium* sp.

*Solidago* sp.

##### *Minor plants*

*Andropogon argyraeus*

*Helianthus* sp.

*Antennaria plantaginifolia*

*Hypericum gentianoides*

*Aster grandiflorus*

*Lactuca canadensis*

*Chamaecrista nictitans*

*Lespedeza augustifolia*

*Chrysopsis graminifolia*

*Lespedeza procumbens*

*Daucus carota*

*Physalis* sp.

*Desmodium acuminatum*

*Senecio Smallii*

*Desmodium* sp.

*Sieblingia seslerioides*

*Erigeron* spp.

*Smilax rotundifolia*

*Eragrostis* sp.

*Smilax Bona-nox*

*Gnaphalium purpurea*

##### *Seedling growth*

Seedling growth is of great importance in regard to the rôle of plants in any sere. While the juvenile growth alone is not an adequate index of ecesis, knowledge of this phase in vegetational studies is paramount.

In the early period a slight shift to the dry soil condition may easily prove fatal. With rapidly growing species, however, the importance of such a change is less significant. Likewise, plants growing in shade may be affected to a less extent at such times.

*Experiments.* Seedlings of aster, golden rod, broom-sedge, and crab grass were placed in competition in several pots of loam soil and subjected to artificial drought. The tall weed and broom-sedge seedlings were 1 month old. The crab grass was less than 2 weeks old. After 1 week with no water the weeds could not be revived. Only 2 of the 6 broom-sedge seedlings survived sufficiently to start growing again. One of 2 crab grass seedlings lived. At the end of the week without water, the leaves of crab grass were only rolled; those of the other plants were rolled and dried. Seedlings in other pots subjected to the same drought treatment, but removed from the direct sunlight, rolled after 2 weeks.

Two pots of 14 day old broom-sedge seedlings were placed in the shade of potted plants in the greenhouse. Two other pots were not shaded. The shaded plants lived 3 days without water. The seedlings left exposed to the direct sunlight died the first day.

Bermuda grass and broom-sedge seedlings were grown in the same pot. The plants were not watered through the day as is necessary for the perpetuation of broom-sedge in the open. The Bermuda grass showed no indication of a drought condition, but the broom-sedge died.

The apparently slow growth of the weeds and broom-sedge is of importance. After 6 weeks of growth the leaves of broom-sedge averaged 2 cm. in length. Tillering was very pronounced at this time. Weeds of the same age were twice as tall. Crab grass in the period of 6 weeks flowered at the height of 25 cm.

*Field observations.* Seedling crab grass and weeds are rarely found before the middle of May. Broom-sedge, on the other hand, may be found in abundance by the first of the month. The oldest seedlings at this time may be about 3 weeks of age. By the middle of May there are very few small seedlings surviving in the bare areas. The rains become more and more infrequent and the critical periods—a few days without rain—come on and only the oldest seedlings are able to endure.

Broom-sedge seedlings literally cover the ground in lowland areas. The frequency with which they occur here is much greater than in the adjoining upland regions. In the latter situation the leaves may roll after a few days with no rain. However, under the same conditions those seedlings which grow near clumps of weeds or under the leaves

of broom-sedge do not appear to be affected. Search for seedlings of this plant in Bermuda grass consocieties revealed not a trace of their presence.

#### THE HABITAT IN RELATION TO THE VEGETATION

The bare sunny areas give rise to those species which are usually photophilous. However, from the standpoint of xerism the exposed subsoil and sandy clay loam soils are occupied by very different pioneers, and changes in the vegetation progress at different rates. Where the surface soil is eroded, leaving the red subsoil exposed, changes in soil conditions must be brought about before the broom-sedge can successfully ecize; or more correctly, the habitat must be made favorable for the successful invasion of the tall weeds. Usually in these areas the nutrient-water complex operates. The only change produced in the habitat of the loam and sandy soils is that induced by the tall weeds. The soil water conditions are much more favorable at this time for seedling establishment. This can be shown to be of vital importance to broom-sedge. However, the influence of texture becomes a factor even in this regard.

Variations in soil and aerial habitats are often clearly shown on slopes. And the vegetation of the old fields shows marked response to the water, texture, and nutrient conditions often present in such areas.

The prisere stages are always altered by the depth of the surface soil. In deep sandy soils the advance is rarely as rapid as in those more shallow

#### *Quadrat studies*

*Bare area quadrats.* Meter square areas were spaded up in various field communities in the early spring of 1931. The vegetation in these quadrats was recorded twice (early summer and fall) during the year.

On a sandy clay loam upland area, Bermuda grass became the dominant element, while ragweed played the rôle of the subdominant. A plot was located in an area dominated by the grass just mentioned. In June 3 ragweed seedlings were growing well in the 20 cm. intra-quadrat. By fall only 1 attenuated plant remained, while 8 grass plants had appeared.

In another Bermuda grass consocieties, bared plots gave rise to a luxuriant growth of crab grass. Smart weed and cockle bur were minor elements. This area was of sandy clay, but in lowground. Crab grass became the dominant element in an upland sandy loam area. Yellow foxtail played the minor part.

The bared plot in an *Andropogon* consocieties gave rise to 43 ragweed and 24 Carolina golden rod plants by June. In the fall 34 ragweeds remained. These ranged from 15-60 cm. in height. At the same time the golden rod numbered 23. Some showed definite suppression. Not all of these flowered. The height of these plants ranged from 10-40 cm. Crab grass was represented by 53 hardy plants. *Lespedeza procumbens*, though reduced in numbers, was still present in the fall. In the 20 cm. intra-quadrat ragweed and *Lespedeza* paid the heaviest toll to competition. Of 6 *Lespedeza* seedlings in June, 2 survived until fall; and of 4 ragweed only 1 survived. All 6 crab grass plants flowered.

The open plot in poverty grass was in contrast to the above mentioned area. This quadrat was located higher on the same gentle west-facing slope. Other than the few inches of sandy clay, the soil is of heavy red clay. The soil habitat is in direct contrast to the lower part of the slope which is of deep sandy clay loam.

By late May, 13 plants grew in the quadrat. One week later there were 114 seedlings. Though *Lespedeza* and ragweed had increased numerically, bracted plantain (*Plantago aristida*) was dominant. In contrast to this there were 400 seedlings in the lower quadrat. By fall *Lespedeza* was reduced from 29 to 6 plants and ragweed from 19 to 10. The mortality of all seedlings was greater in the stony sandy clay soil as compared to those in the loam soil. Crab grass was represented by only a few plants.

Crab grass became the dominant element in the bared areas in *Eupatorium* and *Helenium* consocieties. Bermuda grass and sneeze weed were the minor elements. The soil in these contiguous consocieties was of the sandy clay loam type.

It is commonly observed that where the Cecil clay subsoil appears at the surface the vegetation is scant. Poverty grass and button weed usually dominate such areas. And Carolina golden rod is often scattered over the clay surfaces. For experimental purposes soil from a *Cynodon* consocieties was removed to an "outcrop" of the red clay. Where fertilizer was applied with the transported soil, ragweed and crab grass grew. There were but 3 plants of the crab grass. These plants did not appear elsewhere on the galled area.

*Aristida* consocieties. This particular area had been dominated by poverty grass for a number of years. The soil to the 6 inch depth is of sandy clay. Beneath this depth a heavy red clay sub-soil is found. The entire area is so stony that it was almost impossible to obtain soil point data.

The spring vegetation consisted of the usual hop clover and bracted plantain. This was followed by rabbit foot clover.

In the fall of 1930 four asters flowered. The following spring 16 rosettes appeared, but these were reduced to 8 by the middle of June. At this time the average height of these plants was 10 cm. Only 4 of the 8 plants flowered the next fall. It may be well to call attention to the fact that in deep sandy loam soils aster had reached the height of 25 cm. by the first of May. This contrast is no more striking than that of the rabbit foot clover in various localities. It should be stated that this clover grew to the average height of 20 cm. in this consocieties. Where manure had been applied the plants averaged 45 cm. in height.

*Cynodon consocieties*. This upland area of sandy clay loam had been uncultivated for 4 years. In the quadrat 3 asters flowered in the fall of 1930. Early the next spring 34 rosettes of this plant were growing. By June this number had increased to 38. However, only 7 of this number survived and flowered in the fall. The height of these plants ranged from 25-40 cm. This species of aster (*A. ericoides*) normally grows much higher.

*Aster consocieties*. This area apparently had been in Bermuda grass a few years previously. It indicates directly what will happen in the *Cynodon consocieties* in the next few years. This *Aster consocieties* was on a north-facing slope of shallow sandy loam.

The vegetation in the fall of 1930 was represented predominantly by aster (*A. ericoides*) and Bermuda grass. By May of the following year rabbit foot and hop clover covered the area. A month later the vegetation was changing to aster; Canada lettuce, ragwort, and wild carrot were the minor elements. Bermuda grass was reduced from 23 to 4 plants in the course of a year. During the same period aster was eliminated to almost half of the 1930 growth. Of 57 plants in June, only 14 flowered in the fall. On the other hand, broom-sedge increased from 6 plants to 8 in the course of the year. The tussocks did not develop to great size.

*Solidago consocieties*. The soil condition is the same as that in the *Aster consocieties*. The quadrat was approximately in the same location.

By the middle of May the plants in this consocieties had reached the average height of 25 cm. A month later it was definitely affecting field sorrel (*Rumex acetosella*) and daisy fleabane (*Erigeron ramosa*). Of the 69 weeds present in June, 51 flowered in the fall. Sensitive pea (*Chamaecrista nictitans*), Bermuda grass, and beggar-ticks (*Desmodium acuminata*) were much suppressed. Only the *Desmodium* flowered.

*Syntherisma-Helenium* associates. These plants were the main elements in a pasture. The soil is a sandy clay loam.

In spite of the evenly scattered sneeze weed, crab grass was still dominant in the fall of 1931. The minor element was represented by Bermuda grass. Both dog fennel and broom-sedge were invading this area. However, the former was most aggressive.

*Eupatorium consocias*. This community occupied the deep sandy loam area near the *Syntherisma-Helenium* associates.

There was no change in this area over the one year period. However, it was of interest to note the prevalence of *Lespedeza*, but the absence of crab grass. Sneeze weed was sparingly represented. Broom-sedge was much better represented in this community than in the previously mentioned associates.

*Solidago-Andropogon mictium*. The soil type of this area is a deep sandy loam.

A record of the 1930 growth was not obtained from this area. Of the 38 golden rod plants which appeared in June of 1931, only 21 remained until fall. The broom-sedge maintained its representation of 16 tussocks.

*Euthamia-Andropogon* associates. The sandy clay loam soil of this area is 60 cm. deep.

Other than the changing spring vegetation of hop clover and rabbit foot clover this area remained the same throughout the growing season. Many of the golden rod plants reached only 20 cm. in height. On the other hand, broom-sedge grew to the maximum height of 70 cm.

*Andropogon consocias*. This community was on an upland area of red Cecil clay.

Of the 25 tussocks in the quadrat only 10 flowered in 1930. About half of this number flowered the following year. By June of 1931 several aster plants appeared. These were entirely eliminated during the summer.

#### *Summary of quadrat studies*

The greatest number of a single species that appeared in the bared areas was crab grass. It grew abundantly on lowland and upland loam soils. On the clay soils this grass was meagerly represented. These areas of thin soils gave rise to ragweed and poverty grass.

The persistence of crab grass was observed to be most pronounced in the short weed communities. In areas dominated by tall weeds it was rarely found. Conversely, broom-sedge was best represented in the

tall weed communities. The study of the *Cynodon* consociates indicates the slow ecesis of *Aster*. A further stage of this was demonstrated in the *Aster* consociates in which Bermuda grass was definitely suppressed. The increased representation of broom-sedge in the same quadrat points to the trend of the succession. The establishment of broom-sedge with resultant suppression of weeds was indicated in the *Andropogon-Solidago* quadrat. The competition of tall weeds with broom-sedge seemed to be more intense than that in weed consociates.

From the standpoint of soil conditions, it will be sufficient to point out here, that the succession rate seems to be retarded most on the exposed subsoil. The pioneers on these areas are of the xeric type. On the rather shallow or deep loam soils the usual pioneers are present.

#### *Terraced slope transect*

This particular study will bring out three major influences which retard the prisere. However, the same factors which operate to produce this effect are general and important for all the situations as they exist in the ordinary development of the prisere.

Except for a few areas of exposed subsoil, the field is sandy clay loam from 2 feet to 6 inches in depth. The greatest depth of the surface layer is on the terraces and at the foot of the slope. Below this range is the red subsoil.

The inter-terraces were rather uniformly covered by broom-sedge; the only exceptions being the galled areas on which bracted plantain and poverty grass grew (text fig. 1). Boneset (*Eupatorium* sp.), golden rod (*Solidago* sp.), and cudweed (*Antennaria plantaginifolia*), which usually accompany broom-sedge, were well represented throughout the field. Other minors were beggar-ticks, sensitive pea, tickle grass, Carolina golden rod, aster, golden rod, and dog fennel. At the base of the slope the mesic *Andropogon glaucopsis* was co-dominant with *A. virginicus*. But on the slope this more mesic species thinned out to only occasional tussocks of reduced size. Trailing bramble and other species of *Rubus* were well represented in the low ground. These ranged half-way up the slope and were most prominent on the terraces.

The series of terraces presented a peculiar distribution of old field vegetation. The first of the 5 terraces considered was occupied by Bermuda grass as dominant, with aster (*A. Tradescanti*) subdominant. The upper part of the second terrace was crowned by dog fennel (*E. capillifolium*) (fig. 6). In a small area golden rod (*Solidago odora*) was dominant. On the lower end of this same terrace, which gradually dis-

appeared, was aster. Broom-sedge was dominant where the terrace completely faded out. On the third terrace aster played the dominant rôle in a local area, but broom-sedge was invading this consocieties. Again, broom-sedge was dominant where this terrace leveled to the slope. Broom-sedge, aster, and Bermuda grass occupied the two remaining ridges with bramble, golden rod, and Carolina golden rod as minors.

Thus, where the terraces were still prominent, Bermuda grass and weed associates registered dominance. However, where the ridges faded out broom-sedge—tall weed mictia were found. The ecotone was abrupt on the upper side of each terrace; on the lower side weeds and broom-sedge mingled (fig. 6).

In the spring the terraces were densely covered with rabbit foot clover. This plant was practically confined to the ridges. On the lower side of the "aster terrace" (No. 3) vetch was the spring dominant. The ecotone between this species and broom-sedge was very definite. By fall this area was given over to Bermuda grass with aster only a minor element (fig. 7).

Several changes occurred the following year ('32). Bermuda grass no longer was dominant. It was replaced by aster. The dog fennel on the second terrace gave way to aster also (figs. 8 and 9).

### *Water relations*

The importance of water in plant ecesis cannot be overemphasized. The expression of this factor is closely related to texture. And texture plays a very important part in nutrient relations as well.

*Water supplying power of the soil.* For the measurement of this factor Livingston soil points were used. The points were inserted at the 15 cm. depth and removed at the end of an hour. The following data were obtained from the several communities on the slope. The readings are given in milligrams.

	May 29 (5 days after rain)
Eupatorium consocieties.....	600
Andropogon consocieties.....	710
Cynodon consocieties.....	980
Andropogon-Aster associates.....	930
Aristida consocieties.....	540

*Texture.* The rôle this factor plays in water relations is of major importance. This is particularly true for plants in the juvenile stage. Samples for analyses were taken from the surface and the 15 cm. depth.



TABLE 1

	DOG FEN- NEL	ASTER	BROOM-SEDGE	ASTER	BER- MUDA GRASS	ANDRO- POGON- ASTER ASSOCIES	BASE OF SLOPE
Coarse . . . . .	18.10	13.27*	14.15†	10.00	12.80	9.76	
Medium . . . . .	14.00	5.41	2.94	5.45	4.00	7.33	
Fine . . . . .	12.12	3.50	3.80	7.05	6.42	8.10	
Very fine . . . . .	42.17	48.00	53.70	55.00	52.00	40.15	
Clay . . . . .	13.40	29.56	24.00	22.20	24.00	34.21	
	99.79	99.74	98.59	99.70	99.22	99.55	
Coarse . . . . .	18.30	13.35	12.90	7.00	7.95	9.30	7.00
Medium . . . . .	0.00	4.00	3.30	0.00	3.30	3.00	6.80
Fine . . . . .	3.50	7.20	4.39	6.00	4.60	4.00	12.50
Very fine . . . . .	49.00	53.50	51.80	49.60	55.13	55.00	46.00
Clay . . . . .	28.60	21.85	27.10	37.15	28.80	28.50	27.45
	99.40	99.90	99.49	99.75	99.78	99.80	99.75

\* Near terminus of terrace.

† At terminus of same terrace.

TABLE 2

	ANDROPOGON CONSOECIES	EUPATORIUM CONSOECIES
Coarse . . . . .	13.27	13.40
Medium . . . . .	6.20	7.25
Fine . . . . .	8.54	9.75
Very fine . . . . .	42.31	42.53
Clay . . . . .	29.45	26.65
	99.77	99.58
Coarse . . . . .	7.85	9.25
Medium . . . . .	5.82	8.73
Fine . . . . .	8.43	11.70
Very fine . . . . .	38.65	40.64
Clay . . . . .	38.40	29.22
	99.15	99.54

The tables give in percentages the various grades found. The first set of figures is the surface soil analyses (table 1).

The texture data (table 2) further emphasize the relation of texture

to the ecesis of broom-sedge. In the *Andropogon* consocieties, dog fennel was still subdominant. Joining this area was the *Eupatorium* consocieties with a few broom-sedge tussocks. These communities were not on the terraced slope. Soil for analyses was taken from the surface and 15 cm. depths. The first set of figures (table 1) is the analyses of the surface soil.

### Light

A stop-watch photometer was used to determine this factor. The method of procedure and calculation recommended by Clements<sup>1</sup> was used. A series of exposures was made on a clear day at noon. This gave the standard, or the most intense light values. Exposures were then made in the various communities. All data except that from the *Vicia* socias were obtained in September at one-half the height of the vegetation. The exposure in pine was made by directing the aperture of the photometer so that the light which filtered through the branches would strike the photographic paper.

The relative average values are given below.

	per cent
Dog fennel.....	9.5
Aster.....	12.2
Bermuda grass.....	8.5
Vetch.....	1.7
Pine.....	2.5

### Summary of habitat studies

In the coarser soils capillary activity is comparatively less than in the fine soils. Wells and Shunk<sup>2</sup> demonstrated this to be the case in their studies of the sand hill region of the lower coastal plain. That the water supply is tied up with texture is fairly well demonstrated by the results in the Raleigh region.

The *Eupatorium* consocieties gave the lowest water supplying power on the basis of texture. The soil texture is of a coarser grade than that of the other communities. In the *Cynodon* consocieties and the *Andropogon-Aster* associates the soil point readings approached equal values. The texture is approximately the same for these two communities. The

<sup>1</sup> Clements, Frederic E. *Plant Physiology and Ecology*. Henry Holt and Company, New York, 1907.

<sup>2</sup> Wells, B. W., and Shunk, I. V. The Vegetation and Habitat Factors of the Coarser Sands of the North Carolina Coastal Plain. *Ecological Monographs* 1: 465-570. Oct., 1931.

*Aristida* consociates gave the lowest water supply value. In this case the soil type was compact red clay. The water either penetrates to only a slight extent or it is held more firmly by the soil particles than in other soils. The low reading in broom-sedge may be accounted for by the fact that the soil points were on the border line between the surface sandy clay loam and the red clay layers. In addition to this, all of the communities except the *Aristida* and *Andropogon* consociates were heavily covered by spring plants. In the contiguous *Andropogon* and *Eupatorium* consociates (not on the slope) we notice only slight differences in the texture. But the finest textured soil is in the broom-sedge area.

The significant facts brought out by these data are: the relation of texture to water as indicated in the two *Eupatorium* consociates, and the low water supplying power of the raw red clay soils in the *Aristida*.

The greatest response to the light factor was found in the dense stand of *Aster* on the third terrace. Bermuda grass seemed to be unable to grow to any extent in this situation. However, it is of interest to note that the dense shade of the early vetch had no effect upon the later development of this grass. In open consociates of Bermuda grass growth begins early in the spring. On the other hand, in the vetch community by the latter part of May there were but few plants arising from the underground stems. Apparently after the death of this legume, Bermuda grass grew rapidly.

The aster, dog fennel, and Bermuda grass communities gave approximately the same light values. Nevertheless, the shade produced by the former seemed sufficient to reduce the grass considerably.

The light value under pine is relatively low as it filters through the branches. This effect is minimized by the reflected light which enters under the branches. Broom-sedge shows little response to this factor where the tree vegetation is not crowded.

The density of broom-sedge tussocks is dependent upon the water supply. Since they are a foot or more apart, light could never become a factor. Those plants which ecize in an *Andropogon* consociates require a maximum amount of light, but their water requirement is probably low. Cudweed, beggar-ticks, sensitive pea, etc., are to be found in the otherwise bare spaces between the broom-sedge tussocks. Especially to be mentioned are the pines which constitute the next stage in the sere.

#### *Ecads*

The response of plants to several known factors can often be readily determined by field observations. With the soil water and the light factors playing a decisive part in the establishment and maintenance of

the principal old field elements, it seems desirable to present data concerning the certain responses to factor changes.

*Broom-sedge.* The distribution of the leaves on the flowering stalks were not as abundant nor as large in the *Eupatorium-Andropogon* complex as in the *Andropogon* consociates. However, the coloration was good. The flowering stalks were of the average height but very slender. Further, the number of stalks produced were comparatively few. All of these conditions indicate insufficient water and low light. Water was probably the most influential. The texture studies in this area are helpful in the interpretation of the water-texture relationship. This is especially important in relation to seedlings.

Broom-sedge under pines showed severe suppression. In such instances, it was probably due to root competition since the plants showed no evident elongation response to the lessened light.

*Dog fennel.* The heads of this plant showed definite suppression on the inter-terrace where broom-sedge was dominant. Not only were the stems reduced in size, but those which grew from the rosettes were fewer in number. In many cases the number of stems was decreased and the size reduced over the number at the same rosette of 1930. Where three stalks of the 1930 growth could be counted only one grew in 1931. Suppression is most noticeable where the dog fennel grows very near the broom-sedge tussocks. In areas where broom-sedge has become well represented, a few minutes' search will disclose many such situations.

*Aster.* The effect of competition for water was very noticeable where this representative grew with broom-sedge. The leaves were not so numerous nor large as in the associates. Occasionally several stems grew from a single rosette. This happened only in the more open spaces between the broom-sedge tussocks. Suppression was greatest where the two species grew closely together. Golden rod made a similar response in competition with broom-sedge.

*Bermuda grass.* By contrasting the plants in the consociates with those in the *Aster-Cynodon* mixture the effects of competition become very noticeable. Line transects of 40 cm. lengths in the consociates gave an average count of 8 plants. In contrast to this, transects in open aster gave an average count of only 4 plants. In the dense stand of aster an occasional spear of grass could be seen. These plants had fewer leaves, and the stems were unusually long.

#### DISCUSSION

Inhabitants of an area become established because of adaptations to the conditions present in that particular habitat. The habitats of bare

and covered areas are, of course, very different so far as the water and light conditions are concerned. Evaporation of water from the soil surface on a bare area is much greater than that where vegetation has developed. And the height and density of the vegetation become factors in the maintenance of surface soil moisture. The evaporating power of the air in such instances is decreased due to the check of wind velocity and solar radiation.

The above facts form a basis for the concepts to account for the vegetational sequence in the prairie. A crab grass community is best represented on bare areas. In competition with open communities of annuals it will maintain dominance. We notice that crab grass holds on for considerable time in the short weed community of *Helenium*. And when opened to light, the small-grain fields often become vegetated with this grass. However, in areas where perennials are dominant, though the light is favorable, this grass is not found. Low water during drought periods is probably the limiting factor, because dominant plants are always as fully represented as the habitat and the time permit.

The rapid growth of crab grass is responsible for its ready appearance in cultivated fields. The hydrophilic condition of colloidal particles in the large water-holding cells may contribute a great deal to its maintenance in open ground. The weeds grow more slowly and are more susceptible to drought in the seedling stage. Further, cultivation may hinder the growth of these plants should they appear. Just why tall weeds do not always become dominant the season following abandonment is not certainly known. The general seeding is probably such that they should become dominant at this time. Sometimes this is the case. In other situations the number of tall weeds that ecize the first season are few. This has been observed on both thin stony soil and lowland loam. A second season after abandonment a weed community is usually found. The number of the previous year is increased both by seeding and by the appearance of plants from the stolons of those already established. The resulting shade eliminates the crab grass.

Bermuda grass loses out to tall weeds, because of reduced light, also. This was clearly seen in the transect study. In the *Aster* consocieties, which shaded into the *Cynodon* consocieties, there was a decrease in the representation of the grass. A further stage was shown on the fourth terrace in which broom-sedge and aster mingled and Bermuda grass was a minor underplant. Invasion into Bermuda grass by weeds requires a much longer time than entrance into one of crab grass. This is related to the growth habits of the grass species. The annual crab grass has no chance with a perennial species. Bermuda grass, a peren-

nial, competes successfully with weeds until considerable shade is produced. It is then suppressed.

The invasion of broom-sedge into old fields rests upon the establishment of more mesic conditions induced by the tall weed flora. Pot experiments have shown broom-sedge seedlings to have a slow growth rate and to be very sensitive to a slightly dry soil condition. In the field it has been observed to germinate and grow in the open, but in a few days without rain the seedlings disappeared. However, shaded seedlings withstood the adverse conditions better. The concept advanced here is that the soil in closed tall weed communities does not dry out so rapidly between the spring rains so that broom-sedge can become established. The various situations on the terraced slope intensify this concept. In the finer soils where aster had suppressed the Bermuda grass, broom-sedge was ecizing. On the coarser soils (terrace 2) the broom-sedge was scarcely represented. It can never enter the *Eupatorium* consociates until there is almost daily rainfall through the spring. The coarse, surface soils dry out much too rapidly for the slow-growing grass to become established. This characteristic is probably responsible for the minor representation of *Andropogon virginicus* in the "sand hills." Similar response to texture difference was noted in another *Eupatorium* consociates and the joining *Andropogon* consociates. The water relations as influenced by texture could have been the only factor operating against the uniform establishment of the broom-sedge.

The ecotone between broom-sedge and Bermuda grass is definite where these two grasses inhabit contiguous areas. The former is rarely found with Bermuda grass. The ecotone between a field of crab grass and one of broom-sedge is just as sharp. If the invasion of broom-sedge was due solely to its apparently slow growth in the juvenile stage, the tall weeds and this grass would appear simultaneously. This does not occur. Not until the weeds have become well established does broom-sedge invade. And we notice that it rarely enters communities of annual weeds. This can be explained by the comparative growth of the annual and perennial weeds in relation to their influence on the soil and aerial habitats during the spring season. Annuals do not grow to any extent until the middle of May. Summer annuals probably do not germinate before that time. Thus, the shade produced by these plants is not sufficient to keep the surface soil from drying out before the intermittent drought periods of early summer come on. On the other hand, the tall weeds which arise from perennial roots and reach an average height of 25 cm. by the first of May, create a different moisture condition in the surface soils. The surface soil in plant communities of this

height does not dry out appreciably in the short time between rains. The rainfall from April 26 to June 15 in 1931 averaged 1 rain every 3 days. The growth of broom-sedge under the weed cover is usually possible throughout May at least. The increasing infrequency of rains after this time would tend to reduce seedling establishment. The shade of the weeds introduces another factor which may be advantageous to the broom-sedge. Transpiration from the seedling grass may be reduced considerably. The low light value in shaded communities is apparently of little consequence to the broom-sedge.

Once established under these conditions, the grass is able to compete with the weeds. The root system so thoroughly ramifies the soil that the weeds are finally eliminated. The keen competition broom-sedge offers was indicated in the reduction of the weeds in the aster quadrat. Wherever broom-sedge is found with other herbaceous vegetation the severity of competition may be seen. And it is only under the influence of the tree vegetation that broom-sedge, once established, shows marked suppression.

The exclusion of the many minor species from the tall weed communities is probably due to their intolerance to low light. Many of the same plants that are found in open fields, appear again in broom-sedge communities. Their representation is always of minor importance. Cud weed, boneset, sensitive pea, and Carolina golden rod are never found in closed communities. While not all of these appear in open fields they often are present in broom-sedge fields. That Bermuda grass is a strong competitor for the fullest expression in an area, is shown by the absence of these plants. The habit of growth is probably the limiting factor. Many of them are annuals. The early growth of Bermuda grass might be sufficient to eliminate these plants. Of the perennials, the Carolina golden rod is known to begin growth comparatively late in the spring. Its possibilities of survival are best in broom-sedge, which seems to develop less rapidly than the Bermuda grass. The growth habit and the water factor probably account for the appearance of these plants in the one open community and their absence in the other.

Another influence in the distribution of vegetation, often present in fields, is the galled areas. The nutrient-containing soil is eroded in such instances, and the soil which remains is such that very little water penetrates. Consequently a major part of the water that falls on these red clay surfaces quickly runs off. Hence, the plants growing on the galled areas must be very xeric in nature.

Where poverty grass or button weed is found in abundance it is safe

to conclude that the soil habitat is of an unusually dry, sterile type. These plants play the dominant rôle year after year. They are freed almost entirely from competition with broom-sedge, and only gradually are weeds able to ecize. Even then, the number of these invaders does not increase to any extent over a period of years. When low nutrient and low water factors are involved jointly, the change in vegetation is brought about by improvement of the texture and the nutrient conditions. This, of course, requires a considerable lapse of time.

It should be mentioned that where the subsoil appears on the level, revegetation is more rapid. Nutrient accumulates more rapidly, and more favorable water conditions exist in these situations than on slopes.

#### SUMMARY

1. There are three definite stages in the revegetation of abandoned fields in the vicinity of Raleigh, North Carolina. While there are a few variations in the species' representation throughout the state, the general trend is the same. The pioneer stage may involve several species. On the thin soil, poverty grass, ragweed, and button weed become the dominants. The more fertile soils become vegetated commonly with crab grass. Bermuda grass is less common. The intermediate stage is always represented by the tall weeds. The several species occupy most any soil habitat. It seems that dog fennel is most particular and favors the sandy soils. Broom-sedge becomes established following the ecesis of weeds. Usually the pines follow this grass.

2. Studies of seedling growth showed very clearly why the three stages in the prisere exist.

3. Quadrats established in various plant communities gave an index to the nature of competition and the resultant successional trend in the prisere. A terraced slope transect introduced a number of differentials which were unusually helpful in carrying out the analysis of priseral development. The texture-water complex proved most important here.

4. Soil point data showed no great difference in the water supplying power of the soil in the several communities. The very coarse soil gave a low reading. The soil point data from red clay soils show clearly why the more xeric plants become the pioneers.

5. The light values were relative. There was a considerable response to reduced light in the *Aster-Cynodon* mictium. And where this factor was not involved in annual short weed communities, crab grass held on.

6. Ecads proved valuable as indicators of small variations in the habitats.



7. This study leads to several definite conclusions: The various soil habitats produce no important changes in the priseral sequence. The rapidity with which crab grass grows, and its ability to withstand longer drought periods than the other species, is responsible for its position as the pioneer. The slower-growing weeds can not become established before the year following abandonment. Slow seedling growth and susceptibility to prolonged drought are probably most influential. The modification in the surface soil moisture brought about by the tall weeds makes possible the invasion of broom-sedge. While the series may be retarded for a time, as soon as the tall weeds ecize abundantly enough to decrease the water loss from the surface soil, broom-sedge will become established. Broom-sedge does not enter bare areas, nor areas with short vegetation, because of the sensitiveness of the seedlings to even slightly dry soil conditions. Both crab grass and Bermuda grass are affected by reduced light. Hence, the establishment of weeds means the elimination of these pioneers. The suppression of the weeds is due to the competition set up by the perennial root system of the broom-sedge.

#### EXPLANATION OF PLATES

##### PLATE 18

Fig. 1. *Syntherisma*-*Leptilon* associes.

Fig. 2. *Chrysanthemum* socies in a *Cynodon* consocies.

Fig. 3. An *Andropogon* consocies. Boneset and red-stemmed golden-rod frequently hold on to the end of the prisere.

##### PLATE 19

Fig. 4. Ecizing pines in broom-sedge. The flowering plants at the extreme right are asters. The prisere advance has been held up because of the persistence of Bermuda grass.

Fig. 5. Mature pine forest with broom-sedge thinning out under the influence of the trees.

Fig. 6. The sole occupant of the upper part of this terrace was dog fennel. Notice the reduced size of the plants growing with broom-sedge.

##### PLATE 20

Fig. 7. *Cynodon* consocies on the lower side of the third terrace. Aster became dominant the following year.

Fig. 8. Aster consocies where Bermuda grass was dominant the preceding year.

Fig. 9. Aster consocies on terrace 2, which has replaced a dog fennel (*Eupatorium capillifolium*) consocies in one year.

PLATE 18



1



2



3



PLATE 19



4



5



6



PLATE 20



7



9



## THE SIPHONAPTERA (FLEAS) OF NORTH CAROLINA, WITH SPECIAL REFERENCE TO SEX RATIOS

By ARCHIE D. SHAFTESBURY<sup>1</sup>

### TWO TEXT-FIGURES

Several years ago, I noticed a marked preponderance of females among the fleas (Order Siphonaptera) collected each year by the zoölogy students at Woman's College, Greensboro. However, I assumed that this was likely due to selection of the larger specimens, which were the females, or to capture of the slower ones which might possibly be the gravid females, and so paid no particular attention to the differential sex ratio among these insects until April 1930, when I examined carefully a freshly killed gray squirrel (*Sciurus carolinensis carolinensis*) and found 31 specimens of the squirrel flea, *Orchopeas wickhami*, which included 23 females and only 8 males. Since I had combed the squirrel very carefully, and had, so far as possible, secured all the fleas, large and small, the question was suggested as to whether this apparent differential sex ratio might not be a real one. The result has been the collection and examination, during the past three years, of a great number of fleas, including over five thousand specimens from common animals in various parts of North Carolina.

<sup>1</sup> I am indebted to many biology students at Woman's College, Greensboro, for aid in the collection and preparation of specimens, particularly to Misses Ida Katherine Allen, Maude Ashworth, Annie Laurie Bason, Mary Brummitt, Virginia Cohoon, Catharine Cox, Constance Herritage, Dorothy McGhee, Ferne Mitchell, Iris Nelson, Jessie Parker, Kathleen Parker, and Catherine Taylor, and also to Elmer E. Brown and Frank Brown who have sent to me a considerable number of fleas from North Carolina and other states. I wish to thank Mr. C. S. Brimley, assistant entomologist, North Carolina Department of Agriculture, Raleigh, for opportunity to examine the specimens and use the records at the State Department of Agriculture. I wish to thank, also, Dr. H. E. Ewing, of the Bureau of Entomology, United States Department of Agriculture, Washington, D. C., not only for permission to use records and to examine type specimens and other material in the United States National Museum in Washington, D. C., but also for assistance and helpful discussion. To Professor J. P. Givler, in charge of the biology department, Woman's College, University of North Carolina, and to Dr. E. A. Andrews, Professor Emeritus of Zoölogy, of the Johns Hopkins University, I am grateful for valuable suggestions.



I have found, in North Carolina, eleven different species of fleas representing ten genera. These fleas have been taken chiefly from ten common hosts, including cottontail rabbit (*Sylvilagus floridanus mallurus*), gray squirrel (*Sciurus carolinensis carolinensis*), flying squirrel (*Glaucomys volans volans*), opossum (*Didelphis virginiana*), wharf rat (*Rattus norvegicus*), house mouse (*Mus musculus*), common mole (*Scalopus aquaticus*), house cat (*Felis domestica*), dog (*Canis familiaris*), and chicken (*Gallus domesticus*), the collections coming from 67 of the one hundred counties of this state. An examination of the specimens in the North Carolina Department of Agriculture, at Raleigh, and in the United States National Museum, at Washington, D. C., shows that one of these eleven species has apparently not been previously reported from North Carolina, and that two additional species have been recorded from this state. The entire list follows.

#### Order SIPHONAPTERA

Sticktight or Tropical Chicken Flea—*Echidnophaga gallinacea* Westwood (1875)

Previous records: The records at the North Carolina State Department of Agriculture include specimens from Wilmington, New Hanover County, from chickens and dogs; Brunswick County, from chickens; Clarkton, Bladen County, chickens; Wallace, Duplin County, chickens; Conetoe, Edgecombe County, host not stated; Raleigh, Wake County, kitten and wharf rat; Troy, Montgomery County, chickens; and Henrietta, Rutherford County, from chickens. The United States National Museum has records of specimens from dog, collected June 15, 1931, at Wilmington, by W. E. Merritt.

Present records: I have examined 38 North Carolina collections of this species, which included 995 specimens from 29 counties, distributed as shown in Table I. Their distribution according to hosts was as follows: house cat 497, dog 469, chicken 29.

It is claimed that this species was originally confined to the old world, but has been introduced into tropical America. It has been reported in southern United States, including Texas, New Mexico, and California, and, in 1927, as far north as Cape Henry, Virginia, where, according to records from specimens in the Bureau of Entomology at the United States National Museum, it was reported from chicken. Jordan (1929c) lists a record of specimens taken as far north as New York City, from rat. Some other National Museum records for this species include such varied hosts as Cooper's hawk, rat, coyote, young quail, ground squirrel, mongoose (Hawaii), and horse (Orangeburg, S. C., Nov. 1894).

An examination of the map, Fig. 1, showing the distribution of the records of the sticktight flea from the various counties of North Carolina, indicates that this species is limited to the eastern and southern portions of the state, although collections of fleas have been made in many of the counties farther west, including rather large collections in Guilford and Wilkes counties. This apparently restricted distribution extends somewhat beyond the limits of the coastal plain region of the state, and rather closely approximates the extent of the lower austral life zone which was outlined by C. S. Brimley in 1913, and later discussed by Pearson, Brimley, and Brimley (1919) in their book on the birds of North Carolina. A study of some of the factors influencing the

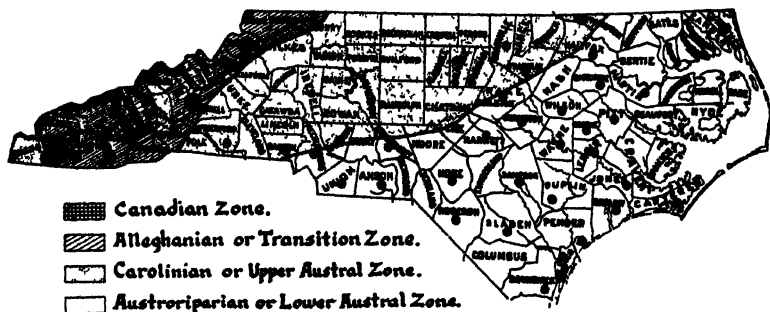


FIG. 1.—Map of North Carolina showing the distribution, by counties, of the sticktight flea, *Echidnophaga gallinacea*, from records (based upon specimens) in the North Carolina Department of Agriculture and in the personal collection of A. D. Shaftesbury. (Base map of Life Zones from "Birds of North Carolina" by Pearson, Brimley, and Brimley, used by permission of J. W. Harrelson, Director N. C. Dept. Conservation and Development )

permanent and perhaps seasonal distribution of *E. gallinacea* might yield interesting results. Is this species gradually spreading toward the north and west, or are there factors which limit the range more or less to what is apparently its present distribution? From the data shown in Table I it will be seen that the few counties which show large collections of this species are well toward the southeastern coast line of the state.

Oriental Rat Flea, Indian Plague Flea—*Xenopsylla cheopis* Rothsch. (1903)

Previous records: State Department of Agriculture—from cotton rat (*Sigmodon hispidus* Say & Ord.), at Raleigh, determined July 29, 1910, by N. C. Rothschild.

Present records: From *Rattus norvegicus*—5 specimens collected Nov. 6, 1931, Raleigh, Wake County; 1 specimen, Jan. 5, 1932, from Greensboro, Guilford County; 1 specimen, Mar. 17, 1932, Greensboro; 3 specimens, Apr. 19, 1932, Greensboro; from *Mus musculus*—2 specimens, Nov. 26, 1932, Raleigh; 1 specimen, Aug. 7, 1933, Plymouth, Washington County.

It is claimed that this is the flea chiefly concerned with the transmission of bubonic plague in the old world, and, according to Fox (1925, p. 133), it "is cosmopolitan in its distribution and is present on the East, South and West coast of the United States. Whether it is present in the interior of the United States is not known." The North Carolina records of this species, though few, offer two points of interest, (1) they are, with one exception, from counties well inland, and (2) a part of them are from the house mouse, which has yielded but few records of any species of fleas.

#### Human flea—*Pulex irritans* Linnaeus (1758)

Previous records: The only record at the State Department of Agriculture was from a deserted house in Buncombe County; records at the United States National Museum include specimens from West Raleigh, Wake County, Apr. 8, 1915, and Halifax County, collected by E. B. Marshall, Aug. 14, 1931.

Present records: I have examined 69 collections of this species from North Carolina, including 1228 specimens from 52 counties, as listed in Table I. The distribution of hosts was as follows: dog 1169, man 39, domestic rabbit (*Lepus* sp.) 8, cottontail rabbit 8, cat 3, gray squirrel 1.

This European species is reported as having a restricted distribution in the United States. Ewing (1929) stated that it was "found particularly in California but occurs sparingly in the Mississippi valley." Later (1931) the same writer mentions the apparently uneven distribution of *Pulex irritans*, also mentioned by Jordan (1929a), and gives a map of the United States showing this irregular distribution, stating that "certain areas exist in which repeated search for this flea has failed to reveal its presence." Dr. Ewing (1931, p. 366) suggests that the absence of records from the southern Appalachian region is probably due to lack of collecting. He recalls that Jordan (1929a) predicted that *P. irritans* would be found in this section. Regarding the coastal plain region, however, Dr. Ewing says that "in his extended work over a period of several years in the eastern parts of the states of Maryland, Virginia, and North Carolina he has never taken a specimen of *Pulex irritans*."

As has been mentioned, I have examined over 1200 specimens of *P. irritans* from 52 North Carolina counties, ranging from Clay and Jackson counties in the west, to Carteret and Currituck counties on the east coast, and, although it varies considerably in abundance in different localities, this species is apparently just as widespread in North Carolina as the cat and dog fleas (*Ctenocephalides felis* and *C. canis*). At present, it could probably be found in any settled section of this state. In this connection, it might be significant to recall my experience with this species in Guilford County. During the years 1924 to 1929, only an occasional specimen of *P. irritans* was found among the 40 or 50 specimens of fleas collected each year by the zoölogy students from cats and dogs about the campus of Woman's College. Since 1929, however,

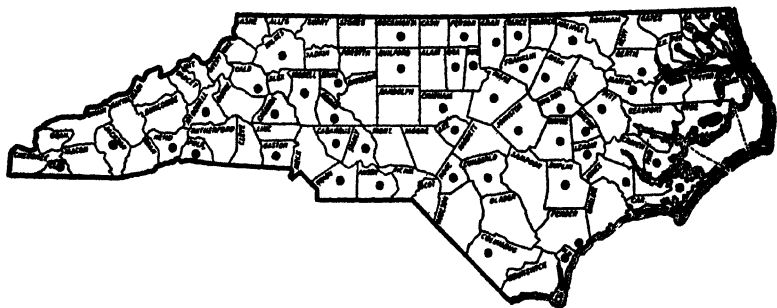


FIG. 2.—Map of North Carolina showing the distribution, by counties, of the so-called human flea, *Pulex irritans*, as indicated by the records of specimens in the writer's collection.

*Pulex* specimens have been present in much larger numbers. From this, it seems possible that *P. irritans* has appeared in the northeastern coastal plain region of the state since Dr. Ewing's field work in that locality, although it must be admitted that Dr. Ewing's collections were from wild animals, and there would probably be little chance of finding a specimen of *Pulex irritans* on them except an occasional accidental stray, since it is a species which infests domestic animals.

*Hoplopsyllus* (Baker, 1905) sp.

Previous records: Specimens of fleas taken from opossum in Raleigh, Wake County, Jan. 10, 1921, by C. S. Brimley and W. B. Mabree, were identified by them as belonging to this genus.

These specimens are now in Mr. Mabee's possession, in Montana, and I have not had an opportunity to examine them. No special opossum flea is known. My own collections from opossums have yielded only a few specimens of the squirrel flea, *Orchopeas wickhami*, and of the cat flea, *Ctenocephalides felis*.

Dog flea—*Ctenocephalides canis* Curtis (1826)

Previous records: A number of records at the North Carolina Department of Agriculture, from scattered counties and various hosts.

Present records: My records for this species include 76 collections from North Carolina, with 863 specimens from 51 counties, as listed in Table I. Distribution of hosts was as follows: dog 804, cat 9, domestic rabbit 1, "dogs and cats" 49.

Cat flea—*Ctenocephalides felis* Bouché (1835)

Previous records: A few in the State Department of Agriculture, of specimens taken at Raleigh and Wilmington, from opossum and dog.

Present records: My records include 74 collections, with 2182 specimens from 49 counties, as shown in Table I. Distribution according to hosts was as follows: house cat 386, dog 1671, opossum 4, man 3, cottontail rabbit 1, flying squirrel 1, "cats and dogs" 31, host not recorded 85.

Although Baker (1904, p. 385) objected to Rothschild's differentiation of *canis* and *felis*, this distinction is now universally accepted. My records show fewer *C. canis* than *C. felis*, although many more dogs than cats were examined. These two species of fleas are apparently found throughout North Carolina, and, though there is considerable difference in their proportional abundance in different localities, *C. felis* is apparently more abundant in most localities, particularly in the eastern part of the state. Both are species which infest domestic hosts, and only an occasional accidental stray specimen would be expected from wild animal hosts. Some of my collection data suggest the possibility of a seasonal difference in the proportions of *C. felis* and *C. canis*, with the former species relatively more abundant during the warmer months of the year.

Rabbit flea—*Cediopsylla (Spilopsyllus) simplex* Baker (1895)

Previous records: Wilmington, New Hanover County, collected from rabbit, by M. Kisiuk, Dec. 22, 1920. (N. C. Dept. Agri.)

Present records: My records for North Carolina include 12 collections, with a total of 189 fleas from 8 counties, as follows—Caldwell 1, Guilford 47, Harnett 1, Mecklenburg 1, Orange 20, Transylvania 1,

TABLE I

THE 1930-33 COLLECTIONS OF THE FOUR MOST COMMON SPECIES OF FLEAS IN  
NORTH CAROLINA, LISTED BY COUNTIES

COUNTY	CTENOCEPHALIDES CANIS	CTENOCEPHALIDES FELIS	FULEX IRRITANS	ECHEINOPHAGA GALLINACEA	COUNTY	CTENOCEPHALIDES CANIS	CTENOCEPHALIDES FELIS	FULEX IRRITANS	ECHEINOPHAGA GALLINACEA
Anson.....		61	3	2	Jones.....	12	123	82	62
Bertie.....	1	96	1		Lee.....		135	3	9
Brunswick.....		18		215	Lenoir.....	1	2	9	338
Buncombe.....	39	30			Lincoln.....	2	30		
Burke.....	3	40			McDowell.....	5		5	
Cabarrus.....	15	25	20	6	Martin.....	30	104	31	1
Caldwell.....	8	47	3		Mecklenburg.....		62		5
Camden.....			2		Nash.....	11	9	31	
Carteret.....	26	9	42	2	New Hanover.....		254	5	10
Catawba.....	12	14	4		Onslow.....		1		6
Chatham.....	1		1		Orange.....	10	1	10	8
Cherokee.....	2	14			Pamlico.....	52	8	67	
Clay.....	3	3	50		Pasquotank.....	5	1	1	
Columbus.....		23	14		Pender.....		96	10	107
Cumberland.....	1	30	1		Perquimans.....	3	20	2	
Currituck.....	192	73	134	2	Person.....	11	21	11	
Davie.....			61		Pitt.....	6		6	10
Duplin.....	1		2		Polk.....	2		4	
Durham.....	6	1	1		Robeson.....	2			1
Franklin.....	3		40	1	Rockingham.....	8	9	12	
Gaston.....	4	27	9	2	Rowan.....	22		3	
Granville.....	4	25	2	4	Sampson.....				4
Greene.....	1	39	14	3	Stanly.....	1	19	1	
Guilford.....	116	315	92		Surry.....	30	2		
Halifax.....	33	4	15	11	Union.....	1	9	2	18
Harnett.....	3	1		24	Vance.....	28	19	2	
Henderson.....	3	4	24	2	Wake.....	4	1	1	
Hoke.....	1		1	46	Washington.....	8	33	4	
Hyde.....		148	20		Wayne.....	6	98	4	9
Iredell.....	18	4	37		Wilkes.....	92	33	325	
Jackson.....	1	41	1		Wilson.....			2	10
Johnston.....	1		1	77	Yadkin.....	13			
					Totals.....	863	2,182	1,228	995

Wilkes 33, and Yadkin 85. Distribution according to hosts: cotton-tail rabbit 153, dog 35, house cat 1.

This is apparently the common rabbit flea of this region, although more extensive collections might prove otherwise. The United States National Museum has records from rabbits in Michigan, Iowa, Massachusetts, Georgia, and Tennessee. I did not find it among a small collection of fleas which I made from cottontail rabbits (*Sylvilagus floridanus*?) and jack rabbits (*Lepus californicus melanotis*) in Stafford County, Kansas, in December 1931.

Mole Flea—*Ctenophthalmus pseudagyrtes* Baker (1904)

Previous records: Some records in the State Department of Agriculture, Raleigh, taken at Raleigh from *Scalopus aquaticus*; a U. S. N. M. record, from meadow mouse, *Microtus* sp., collected by R. L. Boke, on Oconalufy Creek, Swain County, Apr. 18, 1931.

Present records: All from *Scalopus aquaticus*—5 specimens collected July 24, 1932, in Granville County; 3 specimens, Sept. 19, 1932, Guilford County; and 1 specimen, Mar. 30, 1933, Guilford County.

The National Museum specimen records of this species are not very abundant and are from the eastern and central states, chiefly from various species of moles, shrews, and pocket gophers. It will likely be found in the state wherever suitable hosts are found.

Rabbit Flea—*Odontopsyllus multispinosus* Baker (1904)

Previous records: From *Sylvilagus floridanus mallurus*, collected Dec. 7, 1899, at Raleigh, by H. H. Brimley and C. S. Brimley.

Present records: All from cottontail rabbit—3 specimens collected Apr. 6, 1931, Guilford County; 7 specimens, Apr. 13, 1931, Yadkin County; 2 specimens, Dec. 30, 1931, Guilford County; 2 specimens, Apr. 16, 1932, Orange County; and 2 specimens, Apr. 25, 1932, Guilford County.

This species was originally described from one male specimen captured at Raleigh. The few specimens recorded at the National Museum are from North Carolina, Maryland, and Massachusetts, and all are from varieties of cottontail rabbits. Many of the live and freshly killed cottontail rabbits which I have examined in North Carolina have had no fleas of any kind. The fact that, during our collections, 153 specimens of *Cediopsylla simplex* have been taken from cottontail rabbits, while only 16 specimens of *O. multispinosus* have been secured, suggests that this latter species might be relatively more abundant on some other kind of rabbit in some other locality.

Squirrel flea—*Orchopeas wickhami* Baker (1895)

Previous records: One specimen, from flying squirrel, Raleigh, Wake County, Jan. 25, 1927. (N. C. Dept. Agri.)

Present records: I have examined 18 collections from North Carolina, with 370 specimens from 11 counties, distributed as follows—Caswell 11 specimens, Chatham 46, Forsyth 2, Guilford 225, Mecklenburg 3, Onslow 34, Orange 8, Rockingham 12, Union 25, Wake 2, and Wilkes 2. The distribution according to hosts is as follows: gray squirrel 353, flying squirrel 8, opossum 6, and dog 3.

This species, recorded chiefly from squirrels and flying squirrels, has been reported from various of the eastern and central states, and as far west as Arizona. It has been introduced into England with the gray squirrel (Jordan, 1928). It seems likely that it will be found throughout North Carolina, wherever suitable hosts are found. Further collections are needed, particularly in the eastern and western sections of this state.

*Orchopeas leucopus* Baker (1904)

This species, which is closely related to the preceding, has been reported to the National Museum by R. L. Boke from specimen taken in 1931 from the white footed deer mouse, *Peromyscus leucopus leucopus*, on Oconalufly River, Swain County, at an altitude of 3,000 feet.

European and North American Rat Flea—*Nosopsyllus fasciatus* Bosc. (1801)

Previous records from North Carolina: None.

Present records: From *Rattus norvegicus*—1 specimen collected at Greensboro, Guilford County, Mar. 27, 1931; 1 specimen collected at Greensboro, Mar. 16, 1932, by E. E. Brown; 3 specimens from Winston-Salem, Forsyth County, from a rat sent to Greensboro, Apr. 13, 1932, by A. E. Oman, leader, rodent control, U. S. Biol. Survey; 4 specimens collected at Greensboro, Oct. 26, 1932 (from rats infected with a protozoan blood parasite, *Trypanosoma lewisi*); and 3 specimens collected by Miss R. Collie, Raleigh, Feb. 15, 1933 (courtesy C. S. Brimley, N. C. Dept. Agr.)

Interest in the study of the incidence of rat fleas has been added by the recent increase in the number of cases of typhus fever in the southern states. Our endemic typhus is not transmitted by the body louse ("cootie"—*Pediculus humanus*) as is the European typhus. Since the virus of endemic typhus has been recovered from *Xenopsylla cheopis* taken from rats trapped at typhus fever foci, in Baltimore and Savannah, and both *X. cheopis* and *Nosopsyllus fasciatus* readily transmit the



typhus virus from infected to noninfected white rats, they are strongly suspected as being among the agents of transmission of endemic typhus from rats to man. (Cf. Dyer, Ceder, Rumreich, and Badger, 1931; Dyer, et al., 1932.)

Mouse flea—*Ctenopsyllus segnis* Schönherr (1816) (= *Leptopsylla musculi* Duges, 1832)

Previous records: Specimens taken from cotton rat, *Sigmodon hispidus*, at Raleigh, were determined by N. C. Rothschild, July 29, 1910; other specimens, taken from wharf rat at Raleigh, August 1911, were determined by W. B. Mabey and C. S. Brimley.

Present records: Two specimens collected from house mouse at Plymouth, Washington County, Aug. 7, 1933.

Following is a list of the thirteen species of fleas which have been reported from North Carolina: *Echidnophaga gallinacea*\*, *Xenopsylla cheopis*\*, *Pulex irritans*\*, *Hoplopsyllus* sp., *Ctenocephalides canis*\*, *Ctenocephalides felis*\*, *Cediopsylla simplex*, *Ctenophthalmus pseudagrytes*, *Odontopsyllus multispinosus*, *Orchopeas wickhami*, *Orchopeas leucopus*, *Nosopsyllus fasciatus*\*, and *Ctenopsyllus segnis*\*. I have collected and examined specimens of eleven of these species, including all except *Hoplopsyllus* sp. and *Orchopeas leucopus*. These two were added from specimen records at the North Carolina Department of Agriculture, and at the United States National Museum, at Washington, D. C. *Nosopsyllus fasciatus* has apparently not been previously reported from North Carolina. The seven species which are starred (\*) are not native to this section, but are introduced species, and include those most annoying to man and domestic animals, and those most likely to transmit disease.

Jordan (1929a) estimated in 1928 that there were about 800 known species of fleas, of which 131 were known in United States and Canada, the larger number of the North American species being from the western United States. A recent examination which I made of flea specimens in the United States Department of Agriculture, at the National Museum, revealed the fact that there were fewer flea records from North Carolina than from almost any other state. Thirty-one species have been listed from New York (Jordan, 1929c), but, as far as I know, no such definite data are available regarding the fleas of any other eastern state. It should not be inferred that the accompanying list of thirteen species represents anything like the entire number of species of fleas in North Carolina. This list likely represents less than a fourth of the species that should be found in this state.

Other species of fleas which have been collected in neighboring states

and might be expected in North Carolina include *Ceratophyllus riparius*, from nest of bank swallow (*Riparia riparia*) at Roslyn, Va., collected June 1916, by F. C. Bishopp (record from Jordan & Rothschild, 1920); *Stenoponia wetmorei*, collected from a white footed mouse, *Peromyscus leucopus*, in 1927, at Falls Church, Va. (record from U. S. N. M.); *Ctenopsyllus (Leptopsylla) catatina*, described by Jordan (1928) from a specimen taken from an opossum in Pennsylvania; *Neopsylla wenmanni* Rothschild (1904), collected by R. L. Boke, April 1931, from white footed mouse, *Peromyscus leucopus leucopus*, at an altitude of 3,000 ft., at Greenbriar, Tenn. (U. S. N. M.); and *Ctenopsyllus (Leptopsylla) selenis* Rothschild, collected from white footed mouse, *Peromyscus maniculatus*, at Buttry's Cave, Jefferson City, Tenn., Feb. 4, 1933 (U. S. N. M.). *Rhopalopsyllus gwyni*, which was described from fleas of both sexes taken from "rats" at the quarantine station at Brunswick, Ga., in 1904, was believed by Fox (1914) to have come from South America, but numerous specimens of a very similar, perhaps identical, species, *Rhopalopsyllus sigmodoni*, were collected at Houston, Texas (Stewart, 1930), chiefly from *Rattus norvegicus* and the cotton rat, *Sigmodon hispidus*. These records suggest that if *Rhopalopsyllus* is not a native genus, it has perhaps become established in some localities in North America and might be expected from cotton rats and other rats in North Carolina.

The mountain regions of North Carolina have not been examined at all for ectoparasites. Collections from chickarees, fox squirrels, and marsh rabbits have not been made, and examination of such forms as shrews, field mice, and bats would doubtless reveal several species unrecorded for this section, and possibly some new species. A careful examination of the nests of both domestic fowl and wild birds, which has not been made in North Carolina or elsewhere in the Americas, will no doubt yield specimens of both European and American species of bird fleas. Only seven species of Ceratophylli infesting North American birds were listed in 1920 by Jordan and Rothschild. Jordan (1929b) has listed 26 species of North American birds from which fleas have been taken. His own specimens of bird fleas from the United States were taken mainly from the nests of birds in Massachusetts and New Hampshire, and included but few new species. The nests which have yielded fleas in United States include not only some of ocean birds but several of common birds, such as English sparrow, ovenbird, cat bird, wood thrush, robin, and blue bird, so it seems likely that an examination of recently deserted bird nests in North Carolina should reveal at least a few species of bird fleas. A study of the distribution and

predominance of the two species of rabbit fleas throughout the state should be of interest, and a similar study of *Xenopsylla cheopis* and *Nosopsyllus fasciatus*, and other species which might be found on rats should be of value from the point of view of public health.

## SEX RATIOS

The accompanying list (Table II) includes all of the specimens of fleas which I have identified from North Carolina, tabulated according to species and sex. From this table it will be seen that with the six species which have been collected in considerable numbers, there is a decided preponderance of females. The range in variation is from 62.2 per cent females in *Pulex irritans*, to 86.6 per cent females in *Echidno-*

TABLE II  
SEX RATIOS OF FLEAS COLLECTED IN NORTH CAROLINA, 1930-33

	♂	♀	TOTAL	MALES PER 100 FEMALES
<i>Ctenocephalides felis</i> .....	626	1,556	2,182	40.2
<i>Pulex irritans</i> .....	463	765	1,228	60.5
<i>Echidnophaga gallinacea</i> .....	133	862	995	15.1
<i>Ctenocephalides canis</i> .....	295	568	863	51.9
<i>Orchopeas wickhami</i> .....	110	260	370	42.3
<i>Cediopsylla simplex</i> .....	68	121	189	56.1
<i>Odontopsyllus multispinosus</i> .....	3	13	16	
<i>Xenopsylla cheopis</i> .....	7	6	13	
<i>Nosopsyllus fasciatus</i> .....	3	9	12	
<i>Ctenophthalmus pseudagrytes</i> .....	1	8	9	
<i>Ctenopsyllus segnis</i> .....	0	2	2	

*phaga gallinacea*, or, expressed in proportions, from 1 male to 1.6 females in *P. irritans*, to 1 male to 6.4 females in *E. gallinacea*. These figures represent, of course, the sex ratios of collected adult fleas.

Hermes (1923), in tables showing the interchange of hosts and predominance of species, gives figures showing the number of each sex collected for several species of fleas. From California ground squirrels, he collected 2065 males and 2306 females of *Ceratophyllus acutus*, and 86 males and 140 females of *Hoplopsyllus anomalus*. The former gives a ratio of 89.5 males to 100 females, while the sex ratio in the latter species is 61.4 males per 100 females. Hermes collected 117 males and 220 females of *Pulex irritans*, from humans, the ratio in this case being 53.1 males per 100 females. To a part of his Table XV (l. c., p. 326), which

he adapted from McCoy (1909, p. 1014), showing the predominance of species of fleas of the brown rat, *Rattus norvegicus*, in California, I have added the sex ratios, with the results shown in Table III.

The ratios shown in Table III agree, in a general way, with the sex ratios which I have found with fleas from various localities in North Carolina. However, Kopstein (1932) collected 113 male and 79 female specimens of *Xenopsylla cheopis*, from rats in the harbor warehouses of Tjilatjap, Java. This gives a sex ratio of 143.0 males per 100 females, but the proportions in his collections in other localities in Java differed from this, so that the total of all of his listed collections of *X. cheopis* (l. c., pp. 413, 421-422, 426), from Java, was 555 males and 672 females, which gives a ratio of 82.5 males per 100 females. His collections of this species from mountain localities of West Java (l. c., p. 426) alone totalled 341 males and 466 females, which gives a ratio of 73.1 males per

TABLE III

SEX RATIOS OF FLEAS FROM BROWN RATS IN CALIFORNIA (MODIFIED FROM MCCOY, 1909)

	♂	♀	MALES PER 100 FEMALES
<i>Nosopsyllus fasciatus</i> .....	570	1,252	45.5
<i>Xenopsylla cheopis</i> .....	790	1,146	68.9
<i>Pulex irritans</i> .....	225	425	52.9
<i>Ctenopsyllus segnis</i> .....	44	137	32.1

100 females. Kopstein's smaller collections of two other species were: *Xenopsylla astia*, males 76, females 103; *Stivalius cognatus*, males 165, females 219. These give ratios of 73.7 and 75.3, respectively.

These data appear to indicate that the sex ratio of collected adult fleas is commonly below 80 males per 100 females, and, in fact, in most cases, is around 60, or less, males per 100 females.

Sex ratios which differ considerably are found in other animals. Gudger (1906) found a ratio of 3 males to 7 females in *Siphostoma floridae*, a pipefish at Beaufort, North Carolina. Hildebrand (1927), with 103,150 adult top minnows, *Gambusia*, collected in the Beaufort region from June to December, found a decided seasonal variation in the proportions of the sexes, though the females were always predominant, the average ratio being 1 male to 4.4 females. Hildebrand (1933) found among 1442 mature Carolina diamond-back terrapins (*Malaclemmys centrata*) in captivity a ratio of 1 male to 5.9 females. Hill (1926)

with *Platygaster hiemalis* Forbes, a hymenopterous parasite of the Hessian fly, found that approximately 66 per cent of the adults were females. Gershenson (1928) found in the dipterous insect, *Drosophila obscura*, a sex linked factor which caused an abnormal sex ratio of 96 per cent females. These are fairly typical of many records, and apparently in most cases the females predominate. Often the difference is due to a differential birth rate, as is the case with such insects as aphids, ants, and honeybees, where the females, though many are sterile in some of these forms, are strikingly predominant, due to an actually controlled sex ratio of births.

I have been unable to distinguish the sex of flea eggs, and, with the rather limited number of larvae which I have examined, I could not be certain of the species, much less the sex. Careful examination of a sufficient quantity of material would likely obviate this difficulty, but the question would be only partially settled until the effects of temperature, season and weather, kind and amount of food, hybridization, and other factors, which affect markedly the sex ratios in other organisms, have been considered.

There might be a selective mortality, as occurs with many other animals, which might later alter markedly the sex ratios shown at birth. There is a popularly held opinion, which has often been put into print (Geiser, 1923, p. 161; Popenoe, 1926; Wells, Huxley, Wells, 1931, pp. 555-ff.), that in many different groups of animals the males are less viable than the females, that the male is the weaker sex. Very little is known regarding the comparative length of life of the males and females of even the common insects. Hirst (1926), working on the transmission of plague by rat fleas, found that in *Xenopsylla cheopis* and *X. astia* the females live much longer when unfed than do the males, and that a heavy growth of *Bacillus pestis* in the proventriculus causes death in the males much earlier than in the females. With flies of the family Tabanidae, C. S. Brimley informs me, the sex ratios are approximately equal at emergence, but if these flies are caught as they are found in nature, they will give a ratio of many females to one male. With the much investigated common fruit fly, *Drosophila melanogaster*, the sexes at emergence from the pupal stage are about equal in numbers, but Pearl (1928) has shown that on the average the females live longer than the males. (Cf. also, Alpatov and Pearl, 1929.) Female fleas have apparently not reached the high state of economic efficiency of the praying mantis (*Mantis* sp.), which devour the males after mating. The mating of fleas, with the males beneath, has been observed several times, and

Lundblad (1927) noticed the use of the (larger) antennae of the male as clasping organs, but none of the observers has suggested that the male may die as a result of the mating act. Incidentally, does the male flea ever mate with more than one female? If he mated only once, he would be useless thereafter, and might die before the female. What is usual in this regard, with the males of other insects?

Possibly there is no real differential sex ratio, merely an apparent one due to some physical or physiological difference between the sexes. Perhaps the females are required to spend more time on the host in order to provide for the larger amount of nourishment necessary to develop the eggs. Leeson (1932), working on the sex of newly emerged rat fleas (*X. cheopis*), found that all of the females emerged before the males began to emerge. If this is true with other species, then it seems that fleas collected during the beginning of a period of emergence would show a preponderance of females. It has been suggested that the female fleas are larger and are more easily seen, or perhaps they are more sluggish and are more easily captured. Careful dusting and combing of numerous host animals has yielded similar ratios, and has even resulted in the capture of numerous mites smaller than the smallest male fleas. All of which suggests that considerable further investigation must be done before the solution of the riddle of the apparent preponderance of females in the collections of fleas of North Carolina, and other localities, can be even approximated.

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# NEARLY RELATED COPEPODS DIFFERENTIATED PHYSIOLOGICALLY AS WELL AS MORPHOLOGICALLY<sup>1</sup>

(*Cyclops vernalis* Fischer, *C. venustoides*, n. sp., and *C. exilis*, n. sp.)

By R. E. COKER

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## INTRODUCTION

Freshwater cyclopoid copepods are notable in that usually the diagnostic characters are relatively easily observed (as compared with those of harpacticoids, for example) and susceptible of rather precise statement. Nevertheless, in the old major group (Schmeil) of *bicuspidatus-viridis-vernalis*, there occur in various parts of the world so many forms marked by seemingly very small but yet clearly definable differences that much doubt and difference of opinion have properly prevailed as to whether in many cases we have to do with distinct species, or with widely occurring ecological variations or mutations. Such copepods seem to lend themselves well to experimental breeding, and we were particularly prompted to engage in such experiments by discovering at Chapel Hill three forms living in close association and evidently of the same general type, although clearly and immediately distinguishable

<sup>1</sup> Contribution from the Laboratory of Zoology, University of North Carolina, and the Laboratoire d'Evolution, University of Paris. Part of an investigation of copepods aided by grant from the Rockefeller Fund for Research in Pure Science at the University of North Carolina.

by the numbers of segments in the first antennae. Some results of the breeding experiments are treated primarily in other places (Coker, 1933, etc.); but, as a basis for the appraisal of experimental results, it is essential to give adequate taxonomic definition to the types used, and that is the purpose of the present paper. We are able to report notable physiological as well as morphological distinctions.

The copepods in question have, respectively, 17, 12, and 11 segments in the first antennae. Those with 12 and 11 segments are discussed later in this paper. The copepod with 17-segmented antenna we must identify either as *Cyclops vernalis* Fischer or as *C. robustus* Sars, but, by reason of our own observations to be given later, as well as those of Lowndes (1929), we are compelled to place *robustus* in the synonymy of *vernalis*.

#### **Cyclops vernalis Fischer**

*Cyclops vernalis* Fischer 1853

*Cyclops elongatus* Claus 1863

*Cyclops lucidulus* Sars 1863

*Cyclops robustus* Sars 1863

*Cyclops parvus* Herrick 1882

*Cyclops brevispinosus* Herrick 1884

*Cyclops americanus* Marsh 1893

Fischer's *vernalis* was twice redescribed under different names before Schmeil, in his classical monograph of 1893, again redescribed it and identified with it Claus's *elongatus*, having 18-jointed antenna, and with the copepod described by Sars as Koch's *lucidulus*. After Schmeil the species had been well-recognized by students of copepods of Europe, Asia, and North Africa. It seems not to have been recorded as such by American authors, but Sars (1918) placed Herrick's *parvus* in the synonymy of *lucidulus* and Kiefer (1929), rightly we believe, returned *lucidulus*, and *parvus* with it, to the synonymy of *vernalis*. Kiefer also assigned Marsh's *americanus* to the synonymy of Sars's *robustus*, as Lilljeborg (1901) had already done with Herrick's *brevispinosus*, while Lowndes has correctly relegated *robustus* to the synonymy of *vernalis*.

Our copepods conform so closely with *vernalis* in all essential particulars that no extended description need be given. It may be mentioned that we have studied *vernalis* of the vicinity of Paris and have bred them for several generations in the laboratory, so that we are able to make helpful comparisons between the American and European copepods. The accompanying figure of the whole copepod (fig. 1 a) shows the characteristic form of the body, the short 17- or 18-jointed

antenna (17 segments in the right and 18 in the left antenna of the example figure), not longer than the first segment of the body, the outwardly produced postero-lateral angles of the dorsa of the last two thoracic segments (the form of the next to last being particularly char-

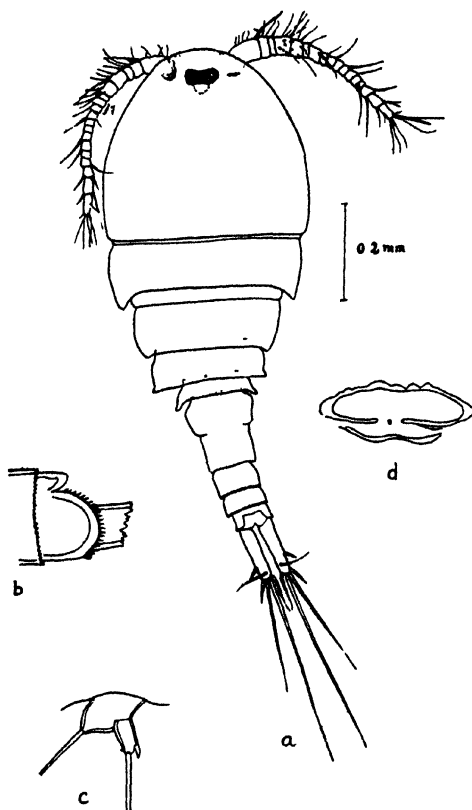


FIG. 1. *Cyclops vernalis* Fischer, Chapel Hill, N. C.: a, adult female with 17-jointed antenna on right side and 18-jointed antenna on left side; b, last abdominal segment and anal plate; c, fifth foot; d, seminal receptacle.

acteristic, since many species have the last thoracic segment produced laterally), the prominent lateral lobes of the genital segment and the form of the furca. The seminal receptacle (fig. 1 d) conforms well with Schmeil's fig. 5 (Pl. II) and Kiefer's fig. 18. *Vernalis* seems to be the

only copepod that frequently has an 18-segmented antenna by division of the 7th segment, as in our example, or, more rarely, by division of the 4th.<sup>2</sup>

The spine formula (for terminal segments of exopodites of  $P_{1-4}$ ) of *vernalis* is supposed to be 2-3-3-3, that of *robustus* 3-4-4-4. In our examples from Chapel Hill the formula is almost always 3-4-4-4 in females, both wild and bred, and 2-3-3-3 in males bred from the same parents as the females with the other formula. Occasionally the reared males have formulas of 3-4-4-4, or 3-4-4-3 (on one or both sides). A few females reared from the same batch of eggs as those with the "typical" armature have the 2-3-3-3 formula, and one had the variant 2-4-4-4 (one side). The European copepods reared in Paris in a pure line starting with a female with formula 3-4-4-4 display that formula in only rather more than half the examples examined; quite a few have the formula 2-3-3-3, and a good many have such aberrant formulas as:  $\frac{3-4-5-5}{2-3-3-3}$ ,  $\frac{3-3-4-3}{3-4-4-4}$ ,  $\frac{3-4-3-3}{3-4-4-4}$ ,  $\frac{3-4-4-3}{2-3-4-3}$  (compare fig. 4, a, b, c). The 2-3-3-3 and 3-4-4-4 formulas may be given by females reared from one batch of eggs under similar conditions of temperature and culture media, but the higher formula is more common in copepods reared at low temperatures, while the lower formula is commonest at high temperatures.

Lowndes (1926, 1927, and 1929) has already shown, by breeding experiments, that the spine formula is so variable in some copepods as to be of slight value for diagnostic purposes, and our observations are confirmatory of this conclusion, although not conforming with his experience that in *americanus* (?) the two forms breed true without giving mixed formulas. Since the alleged specific distinction of *robustus* now rests almost exclusively on the spine formula and an ill-defined difference in the proportions of the basal segment of  $P_5$ , it is not apparent how the more spinous form can be recognized as a species, entailing the identification in two species of copepods bred from the same parents.

The fifth foot (fig. 1 c) is appropriate to *vernalis*, except that the proximal segment is rather wide, as is also true in the Paris copepods; generally it is hardly more than twice as wide as the distal segment. The latter is more slender than shown by Schmeil, but is virtually identical with the corresponding segment in Kiefer's fig. 18 (1929) for *vernalis*.

The proportions of the terminal segments of the rami of  $P_4$  are fre-

<sup>2</sup> We have reared an example of *C. viridis* with 18-segmented antennae, 2 segments representing the normal 7th segment.

quently used for diagnostic purposes, and doubtless properly so within reasonable limits; but these segments show much diversity of form, even in copepods reared from the same batch of eggs and under virtually identical conditions. The end segment of the exopod (fig. 4 a) is commonly broad, being more than half as wide as long. The end segment of the endopod is narrow, but shows notable diversity in proportions. Fig. 2 illustrates two forms found at Chapel Hill, and fig. 3 three forms noted in copepods found or reared at Paris. The quotient of

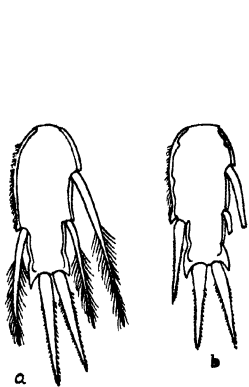


FIG. 2

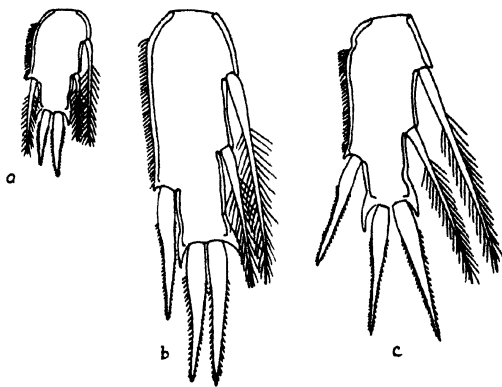


FIG. 3

FIG. 2. *Cyclops vernalis* Fischer, Chapel Hill, N. C., 4th foot, terminal segment of endopod: a, with seta on outer border; b, with spine on outer border.

FIG. 3. *Cyclops vernalis* Fischer, Paris, France, 4th foot, terminal segment of endopod: a, from a female (No. 1513) reared at high temperature, with seta on outer border; b, from wild female (No. 1), with spine on outer border; c, from a large female (No. 1606) reared at low temperature, with spine on outer border. Nos. 1513 and 1606 were progeny of No. 1 (second filial generation). All drawn to same scale.

length into breadth among the latter varied from 0.43 to 0.55 in females and from 0.39 to 0.52 in males. Quotients as diverse as 0.45 and 0.52 were obtained from copepods originating from the same batch of eggs and reared under essentially identical conditions. The outer border of this segment may be armed with a spine (fig. 2 b and 3 b, c) or with a seta (fig. 2 a and 3 a); whatever the armature of the parent, we have found the spine most commonly in copepods reared at low temperature, the seta in those reared at high temperature (Coker, 1934): this was true for the European copepods; we have not had the opportunity as

yet to make similar comparisons for the copepods at Chapel Hill. A spine in this place is one of the supposed distinctions of *Cyclops brevispinosus* Herriek. The 2 terminal spines of this segment are about equal in length, the mesial usually a little longer than the lateral, although sometimes the reverse is the case; the most extreme difference in size is illustrated in fig. 3 a, showing a rare condition.

Schmeil describes the posterior margins of the abdominal segments of *vernalis* as smooth; in our copepods, both reared and wild, at Chapel Hill and Paris, they are commonly inconspicuously dentate, sometimes notably so.

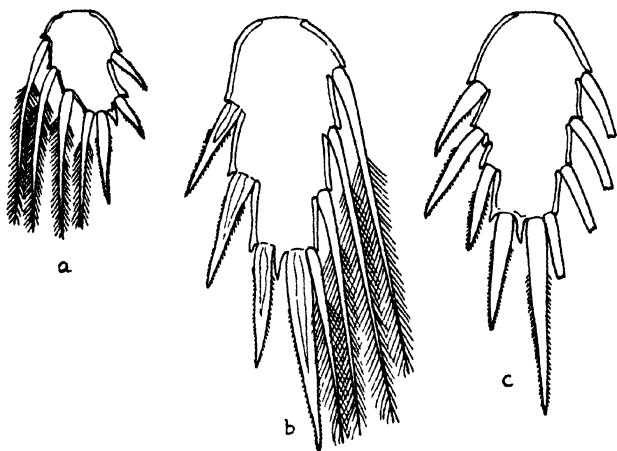


FIG. 4. *Cyclops vernalis* Fischer, Paris, France, 4th foot, terminal segment of exopod: a, from small female (No. 1513) reared at high temperature; b, from large wild female (No. 1); c, from large female (No. 164) reared at low temperature. Nos. 1513 and 164 were progeny of No. 1. All drawn to same scale.

Other figures are given as basis for comparison with the species next to be described. It should be noted that the anal operculum (fig. 1 b) is short, not reaching more than halfway to the posterior extremity of the segment and leaving the sinus largely open above. The furca may or may not show a distinct notch on the outer margin at the end of the basal third, and is without hairs on the inner margin.

Size: The length of *vernalis*, according to both Pesta and Kiefer is 1.2–1.7 mm., females, and 1.–1.2, males. We find that the size of the reared European copepod generally varies inversely with the tempera-

ture prevailing during the period of development, but this subject is treated in another paper. Wild examples taken from one locality at Chapel Hill in 1926, chiefly under ice, had lengths for females, 1.18–1.79, for males, 0.93–1.05 mm. Females taken from another locality during the winter of 1932 had lengths of 0.9–1.30 mm. Copepods reared from these at room temperature had lengths: of females, 0.84–1.09 mm., and of males 0.63–0.74 mm. The furcal rami varied in length from 8 to 14 per cent of the total length of the body, exclusive of the caudal setae (males and females). The rami are usually 4 or 5 times as long as the width at half length. The copepods of this species are generally smaller than those of the following species associated with them.

This copepod was readily bred for several generations in a culture medium consisting of a nearly pure culture of the alga *Ankistrodesmus* making a very green water. It was also bred in infusions of sheep manure or of hay, with *Paramecium*, *Vorticella*, *Colpidium* (?), and other Protozoa. One medium seemed to answer as well as another. Its development at room temperatures is very rapid, complete cycles from egg to egg being effected in several cases in the remarkably short period of 7 days, although sometimes as much as 10 days, occasionally more, was required. Except as to rate of development, the copepod seemed to be almost indifferent to temperature; the rate of development at about 7°C. was about 1/7 that at room temperature. Rates of development were essentially the same for copepods from Chapel Hill and from Paris. As is true of many other species, ovigerous females are often found under ice in winter.

*Vernalis*, although once called a stenothermal glacial relict species (Schokke, 1900), has long been known to be certainly eurythermal and a perennial breeder (Wolf, 1905; Pesta, 1928)—and this affords a point of contrast between it and the species described later in this paper.

Synonymy of *vernalis*, with special reference to *robustus* Sars, *parvus* and *brevispinosus* Herrick, and *americanus* Marsh

Schmeil dealt adequately with Claus's *elongatus* and Sars's re-identification of Koch's *lucidulus*. Sars's *robustus*, described 70 years ago, and redescribed and illustrated by the same author 55 years later, seems not to have been consistently interpreted. As described by Sars (1863 and 1918) it was not distinguished by the spine formula, for Sars recognized that *lucidulus* (= *vernalis*) might have either the lower or the higher formula; it was distinguished rather by the shortness and stoutness of the furca, by the spine on the outer margin of the end segment

of the endopod of the fourth foot and by a few very minor features. Recently, however, it seems to have been the practice to base the supposed distinctiveness of *robustus* primarily on the spine formula, notwithstanding that, if our experience indicates correctly, this would involve associating the name with examples having the furca relatively long and slender, and so doing violence to the original definition. As a matter of fact, the species is not well founded, as Lowndes (1929) has already shown. Lowndes reared typical *vernalis* from parents that were typically *robustus* in spinous armature and in other respects. His only disappointment was that, barring one exceptional case, he could not obtain the *robustus* form from either sort of parent. We have been able to supplement his work, not only by rearing both forms from the same parent, but also by showing that, to a considerable extent, the proportions of the furca, the chief distinction originally, is a function of the temperature at which the copepod is reared (Coker, 1934). It is possible that Lowndes's failure to obtain the *robustus* form in spinous armature from *robustus* parents was due to his care to rear the copepods at the constant temperature of 20°; while the higher spine formula may appear at that temperature, it seems much more likely to occur at low temperatures—7–12°.

Herrick's description of *parvus* (1884) and his figures make the near-identification of that species with *vernalis* inescapable. Why it should ever have been confused with *viridis*, as it was by Marsh (1906) and others, it would be difficult to say. The fifth foot is that of *vernalis*, not that of *viridis*, and the description of the seminal receptacle applies much better to the former species than to the latter, while the fact that the last two segments of the cephalothorax are described as "acute" should exclude *viridis* from further consideration. It may be remarked that, while *viridis* and *vernalis* are probably not distantly related, they are very different in general appearance and in behavior: *viridis* is a heavy-bodied species, relatively slow in movement and very slow and irregular in development; *vernalis*, on the other hand, is light-bodied, nervously active and extremely rapid in development, completing a life cycle under favorable conditions in about one-third the time required for *viridis*—one week, as compared with three weeks or more. Attempts to effect a cross between *viridis* and *vernalis*, either way, have been quite unsuccessful.

We know of no somatic characters to distinguish *parvus* from *vernalis*, but a difficulty remains to be considered. Chambers (1912) reported that American copepods he had identified as *viridis* had 12 chromosomes,



as does the European *viridis*, and that *americanus* had 10, and *parcus* 6. That *americanus* should have 10 chromosomes is as would be expected, for that is the number Braun (1909) found characteristic of *vernalis*; but that *parcus*, which is more typically *vernalis* than is *americanus*, should be ascribed a different number of chromosomes raises the question of identification. There is actually little in Chambers's characterization of the species to identify it with *parcus*. He does not mention the characteristic form of the posterior thoracic segments; he gives the spine formula, 2-3-3-3, which is applicable to many species; he describes the seminal receptacle as having a concave anterior margin and draws it deeply concave like that of *viridis*, which does not conform with Herrick's description—"oval," or with Marsh's "convex" (Marsh, 1920); the fifth foot as sketched is, however, like that of *parcus*. We have thus one positive character to identify Chambers's copepod with Herrick's *parcus*, and one serving the contrary purpose. Whether or not there is significance in the fact that the number of chromosomes found was exactly half of the number possessed by *viridis*, we do not know, but it seems probable that Chambers was dealing with representatives of an undescribed species, intermediate between *vernalis* and *viridis*; we have had some other reasons to suspect that such a species exists. In any event, the chromosome number can be of no taxonomic value except as it may be associated with distinctive somatic characters.

*Cyclops brevispinosus* was described by Herrick as a modified condition of *parcus*, the body and especially the caudal stylets more slender, the outer caudal seta reduced to a short ciliate thorn, the fourth foot modified by great enlargement of the spines and reduction of the setae, the number of the setae the same but "differently disposed," and the form of the seminal receptacle "slightly different;" the fifth foot, as figured, is that of *vernalis*; there seems to be absolutely nothing in the description to afford a basis of distinction from a species as variable as *vernalis* is now known to be. Lehmann (1903) showed that *brevispinosus* and *americanus* could not be positively distinguished. Marsh (1906) emphasized the supposedly distinctive character of the spine on the outer border of the terminal segment of the endopods of  $P_{2-4}$ , but that we find to be common, if not almost invariable, in *vernalis* when reared at low temperatures (Coker, 1934); Lilljeborg (1901) and Sars (1918) have already identified *brevispinosus* with *robustus*.

Among the examples I have reared, *vernalis*, as described by Sars, would seem to be best represented by most of the examples developing at intermediate temperatures, *robustus* of Sars by the occasional examples

developing at high intermediate temperatures that combine low-temperature form with high-temperature size; *americanus* by examples reared at low-temperatures (10°); *parvus* and Sars's unnamed "smaller variety having the caudal rami somewhat shorter" by copepods reared at temperatures above 25°.

We do not suppose that the last word has been said on the taxonomy of *vernalis*, and its varieties, if such they be, but we do feel that the perpetuation of names that correspond with no serviceable definitions renders no useful purpose—but rather the contrary, since the names are necessarily used without agreement as to their significance. We do not concern ourselves now with the 8 varieties of *vernalis* and *robustus* that Thallwitz (1926) has described and that seem to represent a few of the various modifications of spinous and setal armature, some, but not all, of which we have had appear in a single line along with more typical forms. Finally, it may be remarked that Lowndes (1926) has described and figured a form of seminal receptacle which he ascribes to *americanus*, and which he thinks distinguishes that species from *vernalis* and *robustus*; we do not know why the copepods possessing this form of seminal receptacle should be identified with *americanus*, but can express no further opinion regarding them.

#### *Cyclops venustoides*, n. sp.

A copepod found in several places at Chapel Hill, N. C., resembles *Cyclops vernalis* Fischer (1853) in general form but differs from it in several notable morphological characters. It is even more closely related to the comparatively rare *Cyclops venustus* Norman and Scott (1906), with which it may indeed be found to be identical, although there are distinct structural differences between it and that species. We have determined experimentally that there are important physiological distinctions between the copepod in question and *vernalis*, but we have not been able to make physiological comparisons with *venustus*. It may suffice to give figures and a brief structural description by comparison with *vernalis*, point out the few distinctions from *venustoides*, and mention the physiological peculiarities which have been amplified in another place (Coker, 1933).

The anterior portion of the body is smoothly oval, widest near the posterior margin of the long cephalic segment, and tapering uniformly to the last thoracic segment, which is hardly at all wider than the anterior portion of the following first abdominal segment (fig. 5 a). The last thoracic segment is short and markedly produced at the posterior

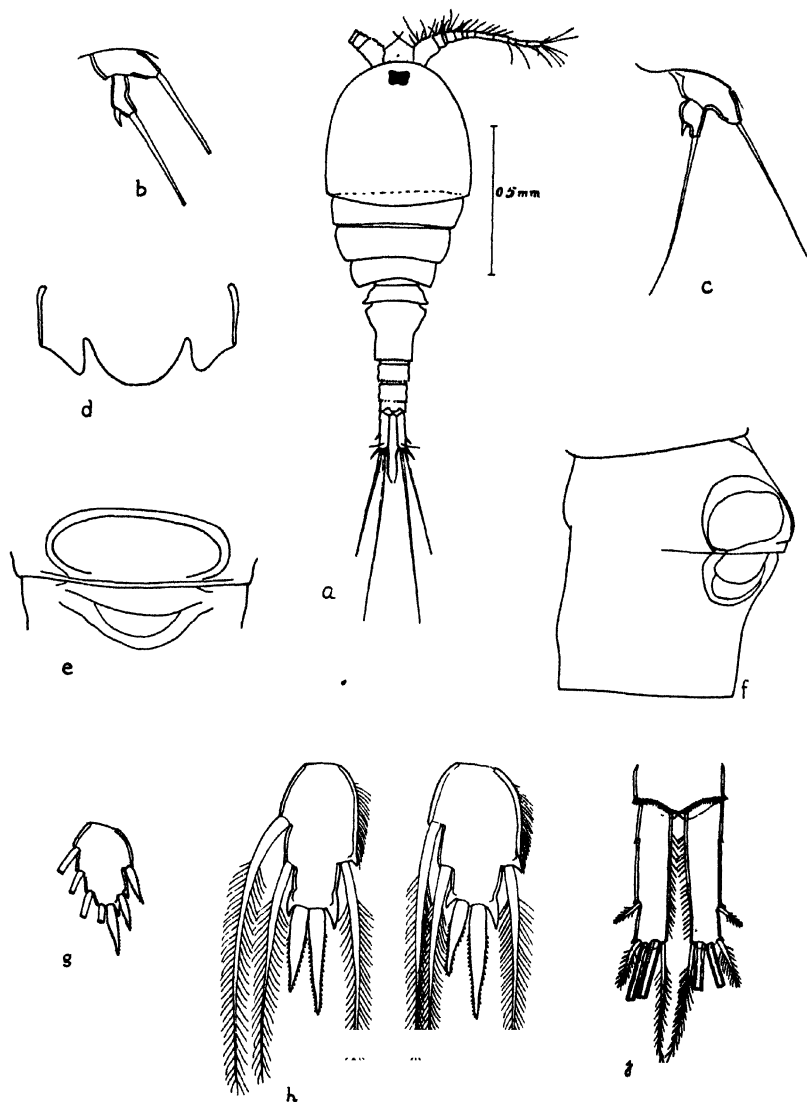


FIG. 5. *Cyclops venustoides* n. sp. (except c): a, adult female; b, fifth foot; c; 5th foot of *C. venustus*, Norman and Scott, for comparison; d, anal plate; e, f, seminal receptacle; g, 4th foot, exopod, terminal segment; h, i, 4th foot, endopod, terminal segment (more highly magnified); j, furca and last abdominal segment, ventral aspect.

lateral angles, but hardly quite as much so as in *vernalis*. The next to last thoracic segment does not have its posterior margins produced laterally, being thus unlike that of *vernalis*. The genital segment, as long as the 3 following segments taken together and expanded prominently at the sides near the anterior margin, narrows rapidly behind these prominences to the line of partial fusion of first and second segments, while that part of the genital segment representing the second abdominal segment has parallel lateral margins. Beyond the genital segment there is a very slight taper to the base of the furca. The anal plate extends far back (fig. 5 d), at least halfway and sometimes fully to the base of the furca, sometimes a little beyond, this being one point of distinction as compared with *vernalis*.

The posterior margins of the abdominal segments, except the last, are conspicuously dentate and fine spinules are observable on the posterior margin of the last abdominal segment. Pronounced dentation of abdominal segments is characteristic of juvenile stages of *vernalis* and therefore this offers a point of relationship. The furcal rami (fig. 5 j) nearly parallel, of approximately uniform width throughout and  $\frac{1}{2}$  to  $\frac{3}{4}$  as wide as long, bear series of fine hairs on the interior margins (their strength exaggerated in the drawing); a slight notch, often armed with a spinelet, is found on the outer margin about  $\frac{1}{3}$  way from back. The "ciliation" of the rami may be thought of as a feature of relationship to *viridis* Jurine. Of the two well developed terminal setae the more mesial is about  $\frac{1}{3}$  longer than the other and rather longer than the abdomen, or from 0.4 to 0.6 the length of the body.

The first antennae (fig. 5 a), about as long as the first segment of the body, are 12-jointed, the first 6 segments and the last 3 being as in *vernalis*, while the relatively long 8th and 9th segments correspond, respectively, to the 8th-11th and the 12th-14th of that species; they show no trace of subdivision. The second antennae and mouth parts seem to be identical with those of *vernalis*.

Both rami of  $P_{1-4}$  are 3-jointed, with spine formula for the distal segments of exopodites 2-3-3-3, in all specimens that we have examined. The full spinous armament is attained in the 4th copepodid stage; the number of segments in the exopodites of the swimming feet increases from 2 to 3 at the molt from the 5th copepodid to the adult, but no additional spines are gained. The distal segment of the exopod of  $P_4$  has width and length in about the proportion of 5:8 (fig. 5 g). The proportions of the distal segment of the endopod of this limb (fig. 5 h, i) have been regarded as of significant diagnostic value; in this copepod

we find them quite variable, the greatest width being usually from 54 to 66 per cent (in one case 51 per cent) of the length, but if the appendage is viewed flat, the length is never "more than twice the width," as is commonly the case with *vernalis* and as is reported for *venustus* by Kiefer (1929). The two terminal spines are always clearly unequal, the inner spine being the smaller, its length  $\frac{1}{2}$  to  $\frac{3}{4}$  that of the outer. The fifth foot (fig. 5 b) is practically identical with that of *vernalis* (fig. 1 c), the basal segment twice as wide as the distal, or a little more, the distal segment narrow, and relatively long, with the spine on the mesial side inserted near but not at the tip, a chief point of distinction between *vernalis* Fischer and *viridis* Jurine (with spine far from the tip).

The form of the seminal receptacle is variable but characteristic (fig. 5 e, f). The anterior portion is much like that of *vernalis*, its anterior margin convex, sometimes flattened or even slightly concave near the middle; posteriorly the organ is more complex than that of *vernalis*, there being, in addition to the usual transverse portion, a deeper (more dorsal) pocket that extends posteriorly in the middle region; the depth and complexity of this pocket is quite variable, but it is always present in some form; the relation of this pocket to the transverse portion and to the anterior portion is not altogether clear. The kidney-shaped spermatophores resemble those of *vernalis*. Egg-sacs are very variable; sometimes there are only 3 or 4 large eggs, loosely attached to each other; sometimes there are about a dozen large eggs in a good sac, and again there may be large well-formed sacs having some 50 eggs in each.

Males are so much like those of *vernalis* that we have not attempted to distinguish those collected in nature. When we have compared males reared from eggs of females of this species with those reared from eggs of examples of *vernalis*, we find the anal operculum, as in females, extending much farther back than in males of *vernalis*, the ciliation of the furca faint or wanting, and the dentations of the abdominal segments inconspicuous. The furcal setae are distinctly longer than those of females, but the longest are not quite  $\frac{1}{2}$  the length of body.

The lengths of females collected in nature were 1.17–1.56 mm., of reared females 1.01–1.13 mm. Reared males were about 0.9 mm. in length. The length of the furca relative to length of body exclusive of caudal setae varied from 0.085 to 0.10, and the width of the furca relative to its length from 0.23 to 0.30; roughly speaking, the furca is about one-tenth as long as the body and each of its branches about one-fourth as wide as long, or a little wider.

This copepod has been found in the vicinity of Chapel Hill, N. C., in two marshy meadows, in one of which we have never failed to find it in winter, and in a spring run in the forest where there was a little water among an abundance of leaves. In each place it was associated with *C. vernalis*.

Type in United States National Museum, No. 69103.

#### Systematic position of *venustoides*

The copepod resembles *vernalis* Fischer in the structure of the 5th foot as well as in the general form of the body. It departs from that species, not only in the number of segments in the first antenna, but also, and we regard this as of more importance, in the form of the next to last thoracic segment which lacks the postero-lateral projections prominent in *vernalis* and seemingly an invariable characteristic of that species. It differs from *vernalis* also in the form of the seminal receptacle, in the possession of conspicuous dentations on the posterior margins of the abdominal segments, wanting or inconspicuous in adult *vernalis* (although quite prominent in juvenile stages), and, generally, in the decided inequality of the terminal spines of the endopodite of P<sub>4</sub>. There is much diversity of form and armature in the terminal segment of that member in both species, but, withal, the relative equality of the terminal spines seems remarkably constant in *vernalis* and the conspicuous inequality as constant in this copepod.

Norman and Scott (1906) described a new copepod, *venustus*, strongly resembling *vernalis*, but having only 12 segments in the first antenna. The original description is not complete, and some of the differences between our copepod and theirs seem of minor importance. In ours the distal segment of P<sub>1</sub> is much longer and narrower than shown and described for *venustoides*, and more irregular in outline. The authors show no spinule or notch on the outer margins of furcal rami, but the notch is often quite inconspicuous in our copepods; they make no reference to abdominal dentations, but these are reported by Kiefer (1929) and Dr. Gurney informs me by letter that the dentations are a chief point of distinction of *venustus*. Kiefer gives the spine formula as 3-4-4-4, instead of 2-3-3-3 as in ours, but this is probably of slight taxonomic significance. Figure 1 of Norman and Scott indicates that the anal plate extends relatively far posteriorly and that the rostrum is quite prominent, features in which our copepod conforms with theirs.

Through the courtesy of Dr. Gurney, I have had the opportunity to examine an adult female and some immature examples of *venustus* from Great Britain. The adult female conforms with our copepod and differs

from *vernalis* in the conspicuousness of the dentations of the abdominal segments, the ciliation of the mesial margins of the furca (possibly in the form of the seminal receptacle—this is not clear), in the proportions of the terminal segment of  $P_4$  endopod ( $W/L = 0.52$ ), and in the posteriorly placed anal plate. It differs from *venustoides* in the spine formula (not important) and more notably in the form of the next to last thoracic dorsum, which is like that of *vernalis*, and in the relative lengths of the terminal spines of  $P_4$  endopod, which are as in *vernalis*. Furthermore, the distal segment of the 5th foot is much wider than in our copepod; fig. 5 c, drawn from the example in hand conforms well with the original figure and description of Norman and Scott; the length of the segment is not over  $1\frac{1}{2}$  times the width, whereas in ours it is twice the width. The longest furcal seta, while not nearly equalling the length of the body, as noted by Kiefer, is much longer than in ours: its length is 71 per cent of that of the body, while in ours it is 40 per cent to 60 per cent.

*Venustus* is evidently intermediate between *vernalis* and *venustoides*. We need not altogether dismiss the possibility that both *venustus* and *venustoides* are varieties of *vernalis*, or that *venustoides* is a subspecies of *venustus*, but, measured by the best existing taxonomic criteria, each is clearly a distinct species. Furthermore, we have found that *venustoides* breeds true and that it is very different from *vernalis* physiologically.

#### Physiological peculiarities of the species

This copepod at Chapel Hill is found regularly associated with those we have identified as *vernalis*, but the two species react very differently to conditions of temperature. *Vernalis* develops very rapidly at room temperatures ( $23^{\circ}\text{C}.$ , cir.), completing a life cycle, from egg to egg-bearing female in 7 to 10 days. At about  $7^{\circ}\text{C}.$  the female attained maturity in 65 days. What is evidently the same species reared at Paris completed a life cycle in 11 days at  $22^{\circ}$ , and, at an average temperature of  $9.5^{\circ}$  attained maturity in 30–50 days, a close enough correspondence with the copepods of the same species at Chapel Hill. *Venustoides*, reared at Chapel Hill simultaneously with *vernalis* and in identical culture media, took 3–5 times as long to attain the 4th copepodid stage, and, in all our experiments, failed to complete development at room temperature, although the 4th copepodid stage lived even better at relatively high temperatures than did those of *vernalis*. At about  $7^{\circ}\text{C}.$ , and possibly at somewhat higher temperatures, it completes development by molts at long intervals. We have not had viable eggs

or viable early nauplii except at low temperatures. *Venustoides* is, then, distinguished from *vernalis* in that, while the latter is broadly eurythermal at all stages, the former is eurythermal only at certain stages, being narrowly stenothermal in respect of development through the last two copepodid stages. More complete data regarding these experiments are given in another place (Coker, 1933). From its rate of development and response to temperature, *venustoides* must be supposed to be monocyclic, or at most dicyclic, as contrasted with highly polycyclic (or acyclic) *vernalis*.

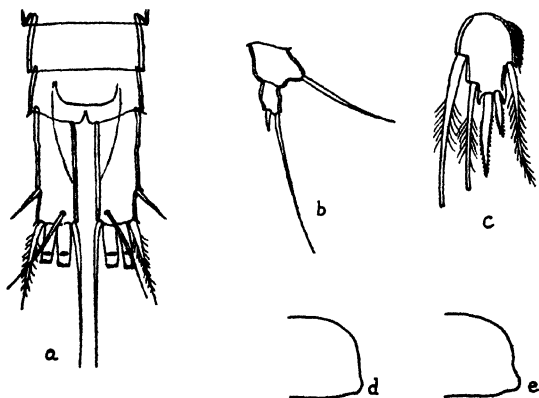


FIG. 6. *Cyclops exilis* n. sp. (a) furca and posterior segments of abdomen; (b) fifth foot of female; (c) terminal of endopod of fourth foot; (d) lateral margin of next to last thoracic dorsum; (e) same, an extreme case of prominence.

### *Cyclops exilis*, n. sp.

In spring runs, both at Chapel Hill, N. C., and at the Allegany School of Natural History in Cattaraugus County, N. Y., there occur in considerable numbers egg-bearing copepods, very similar to *venustoides* in form of body and antenna, but much smaller and generally with 11 segments (occasionally with 12) in the antenna. The missing segment is the short third segment characteristic of all copepods of the group having 12 or 17 segments. In one example the second joint is incomplete, so that, while 11 segments may be counted on the anterior margin of the antenna, only 10 may be counted on the posterior margin. The examples from Chapel Hill, which have been studied more closely, have most of the characteristics of *venustoides*, as to form of seminal



receptacle (although this is very difficult to make out clearly), fifth foot, margins of thoracic segments, anal operculum, relative lengths of caudal setae, etc. They differ *obviously* from the species chiefly in characters that might be thought to be associated with dwarfing, and which we do not, therefore, like to regard as having either specific or subspecific significance. The segments of the antennae, especially the distal six, and all segments of the swimming feet are short and relatively broad; the parallel furcal rami are likewise short and broad: length of furca is 0.07–0.08 length of body, and ratio of width to length of each ramus is 0.38–0.42. The mesialmost apical furcal seta is very slender

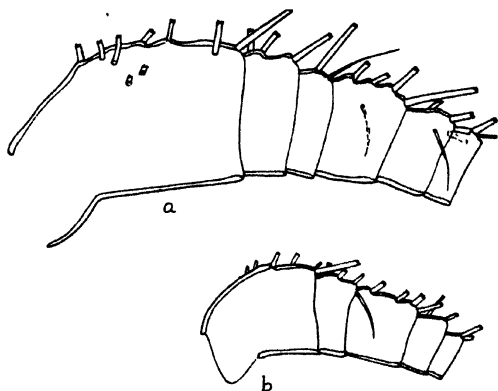


FIG. 7. Proximal segments of antennae of—(a) *Cyclops vernalis* Fischer, and (b) *Cyclops exilis*.

and long, nearly twice as long as the outermost, which is about two-thirds as long as the furca (fig. 6 b); of the two well developed apical setae, the inner is nearly twice as long as the outer, which is about as long as the abdomen. Relative lengths of the apical setae are about as in *venustoides*, but the setae are much longer relative to length of body. The postero-lateral angles of the next to last thoracic segment are not distinctly turned outward, as in *vernalis*, nor so well-rounded as in *venustoides*, but there is generally a more or less definite suggestion of prominence at this angle (fig. 6 c). Interior margins of furcal rami, unlike those of *venustoides*, are bare. Posterior margins of abdominal segments are scarcely if at all dentate.

Experience in rearing *Cyclops vernalis* under different conditions of temperature (Coker, 1934 and 1934a) would readily lead us to suspect

that most of the differences so far mentioned might be attributable to conditions prevailing during development, that the form is an ecological variant; but there is a more decisive point of structural distinction. The two apical spines of the endopod of  $P_4$  are just as unequal as in *venustoides*, but the relations are exactly reversed: the *mesial* spine is invariably the longer. It is conceivable that this difference is attributable primarily to environmental rather than to genetic conditions and we had hoped to submit this question to experimental test. Unfortunately, although females form or carry egg-sacs freely in laboratory cultures, we have not so far been able to rear the copepod at low, intermediate or high temperatures; this copepod has not responded favorably to cultural conditions that have given results with the other species.

There seems to be no alternative but to give the form a name, whether as species or as subspecies seems relatively unimportant. We have collected the copepod year after year and the characters are quite consistent; we have found no examples making the transition to *venustoides*, the form that it most resembles. The larger copepod, with the 12-jointed antenna and ciliated furca, always has the inner apical spine of  $P_4$  endopod decidedly shorter; the smaller form with 10-12-jointed antenna (almost always 11-jointed), with dwarfed appendages and dwarfed and non-ciliated furcal rami, always has the inner apical spine decidedly longer. We apply the name *exilis*, meaning dwarfish.

Length: Females, Chapel Hill—0.78-0.85; New York—0.88.

Mrazek's *michaelseni* seems to have the lateral furcal spine more proximally placed, the furcal rami divergent and a different form of seminal receptacle. Chappuis's *kieferi* has the mesialmost apical furcal seta short and spinelike, instead of notably longer and slender. Chappuis's *reductus* has the rami of the first three swimming feet 2-jointed, instead of 3-jointed. (See Kiefer, 1929). Only further breeding experiments could determine the exact status of the several forms here considered, whether they are all species or only ecological variants of one species.

Type in United States National Museum, no. 69104.

#### SUMMARY

Copepods originally identified as *Cyclops americanus* Marsh from Chapel Hill, N. C., and others identified as *C. robustus* Sars from the vicinity of Paris, France, have been reared for several successive generations in each case. Comparisons have been made with wild copepods

from the regions of Chapel Hill and Paris. The diversity of forms obtained in breeding compels the identification of both sets of copepods with *C. vernalis* Fischer. *Cyclops parvus* Herrick and *C. brevispinosus* Herrick are also assigned to that species. In part this is confirmatory of the views of some European investigators, although it is contrary to existing American practice.

There is described from Chapel Hill a new species, *C. venustoides*, intermediate perhaps, between *vernalis* and *viridis* Fischer, but differing markedly from both. It is nearly related to *C. venustus* Norman and Scott, which appears to be intermediate between *vernalis* and *venustoides*. Another form, with (generally) 11-segmented antenna and otherwise consistently distinct from *venustoides* (with 12-segmented antenna), is provisionally designated as a new species, *exilis*.

*Vernalis* breeds in laboratory cultures and matures with great rapidity at room temperature, the cycle from egg to egg being completed in 7-10 days; a period about 7 times as long is required at a temperature of about 7°C.

The period of development for *venustoides* seems to be 3-5 times as long as for *vernalis* at corresponding temperatures. Furthermore, while *vernalis* is broadly eurythermal at all stages of development and *venustoides* equally so at intermediate stages, the eggs and early nauplii of the latter species lived only at low temperatures in our experiments and arrest of development in the 4th copepodid stage (10th larval stage or second from last) was indefinite except at low temperatures, as we had previously reported before the species could be described and named. Although generally broadly tolerant as to survival at all ordinary temperatures, *venustoides* displays a peculiar sort of stenothermy as to development through certain stages.

The form described as *exilis* has not yet responded favorably to any attempts to breed it in the laboratory.

The group of which *Cyclops vernalis* Fischer is the type remains a very complex and puzzling one. A question that originally prompted our experiments was this: to what extent are the many slightly different forms described as species, subspecies or varieties attributable to extraordinary plasticity within a species or a tendency to explosiveness, genetically speaking? The experiments and observations, for which only the taxonomic aspect is reported here, have thrown some light on the subject, resulting in some simplification and tending to the elimination of names; but it has also added new names, temporarily, at least.

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## A ROT OF PEAR CAUSED BY THE RED BREAD-MOLD FUNGUS

By MINERVA WAYNICK

TWO TEXT-FIGURES

The red bread-mold fungus, *Neurospora sitophila* Shear and Dodge, has long been known to infest bakeries in France, Italy, Germany, and the United States. As a result of such infestations this mold has been extensively studied. As additional habitats, it has been reported to occur on such substrata as on burned tree trunks and on various fruits. Apparently, however, no studies have been made of it as a fruit-rotting organism. It seemed desirable, therefore, when this organism, in its conidial stage, appeared as a soft rot of pear fruits obtained on the market in Durham, North Carolina, to make a study of its parasitism and the nature of the decay which it incites.

Pure cultures were first obtained from the growth resultant from transferring to potato agar, tissue from decaying pears. Subsequent isolations of the same organism were made from other decaying pears and from the surface of pots of sand which had been autoclaved and on which the fungus appeared fortuitously. Identification of the causal organism as "Sex B" of his monilioid, pigmented form of *Neurospora sitophila*, was made by Dr. B. O. Dodge.

In order to secure further evidence of the ability of this mold to rot fruits, pears and apples were disinfected with a solution of calcium hypochlorite and placed in moist chambers. The inoculum, consisting of hyphae and conidia, was introduced through incisions made with a sterilized scalpel. Some of the inoculated fruits were stored at a temperature of 20°C. and others at 35°C. Brown, decayed areas surrounded the points of inoculation on the pears after two or three days. For some unknown reason, no lesions appeared on the apples. Within the next few days a loose, orange-colored mycelium, bearing masses of conidia covered the surface of the lesions (Fig. 1). Reisolations were very readily accomplished.

Diseased tissues, when teased apart in a drop of water or when subjected to pressure under a superimposed cover glass showed that the

pulp cells tend to separate intact and that the mycelium is intercellular. These observations were verified by examination of paraffin sections cut at a thickness of 40 microns. Normal pulp cells of pear fruits consist of large, succulent parenchyma cells interspersed between groups of stone cells (Fig. 2, A). In sections of diseased tissue the hyphae may

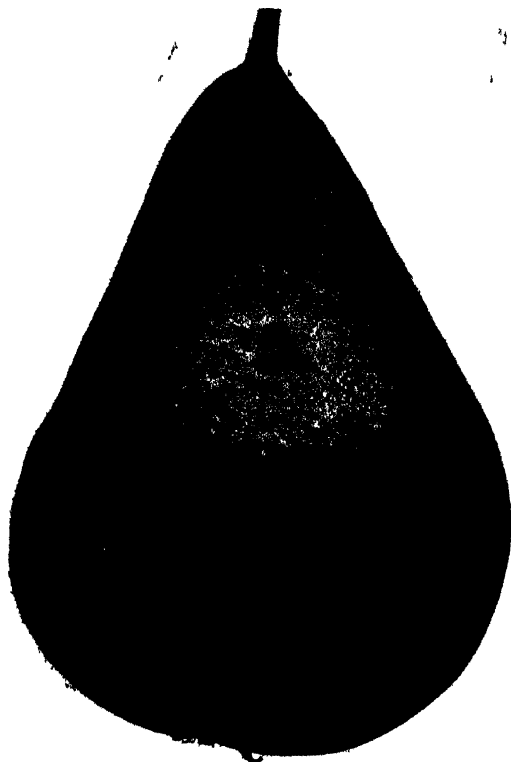


FIG 1. *NEUROSPORA SITOPHILA* ON DECAYING PEARS

be observed to course between the parenchyma cells and to be so abundant as to more or less completely outline the cells in the invaded areas (Fig. 2, C). Evidence of intracellular invasion was noted. The fungus causes a dissolution of the middle lamella slightly in advance of the invading hyphae, which results in the separation of cells (Fig. 2, B).

Following this, the cell walls disintegrate and the cell contents are utilized by the parasite. These contents appear to consist mainly of reducing sugars, as indicated with Benedict's test, and of organic acids. Starch is absent.

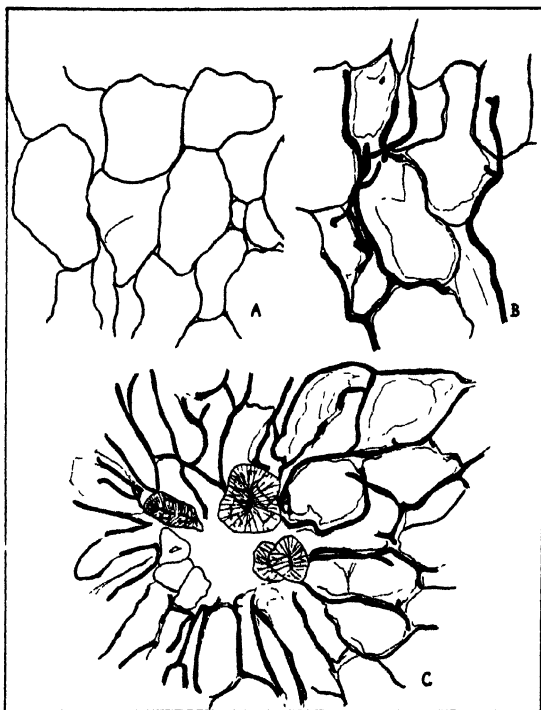


FIG. 2. SECTIONS OF TISSUE FROM PEAR FRUIT

(1) Normal uninfected cells. (2) Intercellular mycelium among cells whose cytoplasmic layer has shrunk, and whose walls are being dissolved. (3) Cells outlined by intercellular mycelium.

A study of the activity of *N. sitophila* in culture was made to confirm the microscopic findings. When soluble starch in starch agar constituted the sole nutrient, the fungus made a sparse growth and there was no evidence, as shown with iodine, that appreciable quantities of starch had been utilized in cultures one week old. Potato agar, however, supported a luxuriant growth. Transfers of hyphae to this medium



resulted in growth that bore new crops of conidia within 4 or more hours. When the initial reaction was pH 7.4 to 7.6 in tube cultures on potato agar and brom thymol blue was used as an indicator, a broad yellow zone formed at the surface of the agar within 24 hours, indicating production of acid. As growth continued this yellow zone extended to the bottom of the tube.

Mycelial growth is not confined to the surface of the agar but is almost equally luxuriant within the agar to a depth of 15 to 20 cm. When melted cooled tubes of slightly alkaline potato agar are inoculated with conidia and are then agitated to distribute the inoculum throughout the tube, mycelium develops throughout the entire length of the tube, and the reaction changes to acid within two or three days. This fungus seems to grow well as a facultative anaerobe—a fact which has been previously noted and which is worthy of critical study.

Acid formation occurs both in 1 per cent dextrose and in 1 per cent sucrose agar when the initial reaction is alkaline, showing that both sugars can serve as food for this mold. When the medium containing either of these sugars is slightly acid at time of inoculation, a blue layer forms just at the surface of the medium and persists for a week or more.

Pectin, one of the pectic constituents in pears, does not appear to be utilized in quantity. This was shown by failure to secure marked increase in acidity resultant from growth on alkaline pectin agar. Pectin agar was prepared according to a method used by Wolf<sup>1</sup> in which "Certo" was purified by repeated precipitation with alcohol. It is very apparent, however, as shown by examination of tissues, that the primary membranes of the pulp cells, which are constituted of calcium pectate, serve as a food substance. Presumably protopectins and certain of their cleavage products can also be utilized.

#### SUMMARY

*Neurospora sitophila* causes a soft rot of pears on the market. Decay is accomplished by dissolution of the primary membrane, after which the cell contents are utilized by the parasite.

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<sup>1</sup> Wolf, F. A. Studies on the physiology of plant pathogenic bacteria. VI. Pectic fermentation in culture media containing pectin. *Phytopath.* **13**: 381-385. 1923.

# NOTES ON THE EGG-LAYING AND NESTING HABITS OF CERTAIN SPECIES OF NORTH CAROLINA MYRIAPODS, AND VARIOUS PHASES OF THEIR LIFE HISTORIES

By WILLIAM S. CORNWELL

The data for the following notes on the egg-laying and nesting habits and various phases of the life histories of certain species of Myriapods were taken at various times during the months of June and July, 1933, by various collectors from the Zoology Department of Duke University. Most of the evidence was obtained from the Duke Forest although some of it came from the vicinity of Durham, N. C., outside the Duke Forest and from elsewhere in the state of North Carolina.

## JULIDAE

*Spirabolus marginatus* (Say). Collected by Dr. A. S. Pearse, June 3, on Bent Creek near Asheville, North Carolina. Two nests were found. In one, located under a log, a big *Spirabolus* was lying on top of 480 separate cocoon pellets, of which each of those examined proved to contain an egg. In the other nest, also under a log, 90 cocoon pellets were discovered. The nests may be described as holes in the ground with no upper covering of earth, the body of the female and the logs under which the nests occurred serving that purpose.

The cocoon pellets, which were dark brown in color, measured 5 mm. by 4 mm. They seemed to be composed of organic soil material or possibly excrement. The spherical white eggs were very small in comparison with the pellets, measuring only 1 mm. in diameter.

Additional evidence of the egg-laying activities of *Spirabolus marginatus* was found in a swampy area adjacent to the Eno River, near Durham. Here the nests of *Spirabolus* seemed to be located in the logs themselves. Several of these animals were found in cavities underneath the bark of greatly decomposed logs. In numerous cases many pellets were found which apparently contained no eggs and seemed to be only excrement. Other pellets of similar nature did contain eggs. These observations were made in late July.

## SCOLOPENDRIDAE

*Scolopendra viridis* Say. At different times during the months of June and July, five females of this species with eggs were found in the Duke Forest or in the vicinity of Durham. On June 27, a female with 40 eggs was found by Mr. N. E. Rice in a cavity in the cambium layer of a somewhat decomposed oak log. The nest may be described as open. Apparently the female had made use of an insect passage way in the wood, doing nothing itself by way of construction. The eggs were attached in a mass to the abdomen of the extended female. A gelatinous matrix of very thin consistency seemed to hold the eggs together and in turn attach the mass to the female's abdomen. These eggs were a little larger than those described for *Spirabolus marginatus* and were ovate rather than spherical; length, 2 mm.; width, 1.5 mm.; color, light yellow.

A second female with 46 eggs was collected on June 29. Again the nest, in a partly decomposed and very large oak log, was located on the upper side beneath the cambium bark. The position of the eggs under the abdomen was the same as previously described but the female was curled up. The temperature of the log both under the bark, which was somewhat shaded, and in the cavity in which the nest was located was 27°C.

The other three females with eggs were taken by Mr. R. G. Taylor. The first, July 3, was a small female with 26 eggs. It was found underneath a quite decomposed loblolly pine log. Again the eggs were attached to the abdomen. The other two females, both of which were of small size as compared to the first two mentioned had 19 eggs and 30 eggs, respectively, attached in masses to the abdomens of the females. These females, with eggs, were also taken from under the bark of loblolly pine logs. The size and color of the eggs were the same as previously mentioned.

On July 27, 1933, Mr. R. G. Taylor found a female with 19 eggs attached to the abdomen, and 26 young clasped on her ventral side. The female was observed in a small cavity underneath the outside bark of a loblolly pine log. The young were white in color and about 6 mm. long. They appeared to have the complete number of legs, which is 21 pairs. The eggs were even somewhat more oval in outline than those previously described but were of about the same size.

## CRYPTOPIDAE

*Cryptops hyalina* Say. On July 5, a female with 9 eggs was discovered beneath a not greatly decomposed loblolly pine log. The nest was on the outside of the bark between the log and the ground. In it the temperature was 25°C. The eggs were in a mass next to the bark and the female was curled up on top of them. Minute in size, and oval in shape, these eggs average about 0.75 mm. in length and about 0.5 mm. in width. The color is light brown.

On June 15, two females with young were collected in the Duke Forest. The first found was curled around 9 young on the underside of a loblolly pine log beneath the outside bark. The second was found beneath the bark on the side of an oak log. This female was curled around 10 young.

On July 8, while making an investigation for the occurrence of Myriapoda in the soil, a nest of a cryptopid was found in the forest floor just beneath the humus layer of a loblolly pine standing in the Duke Forest. There were 9 young present all curled up together, but the female was absent, perhaps driven away by the digging operations. The texture of the soil in which the young were discovered was silt loam. The litter above them was composed exclusively of pine needles. The nest can be described as nothing more than a cavity in the soil, showing no apparent activity on the part of the female towards nest construction. While absence of the female prevented accurate identification of the species, the young so far as the observations could be carried were identical with those described for *Cryptops hyalina*.

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# SPIROCHETES IN THE CAT WITH SPECIAL REFERENCE TO THOSE OF THE ALIMENTARY TRACT<sup>1</sup>

By ELOISE E. GREENE

PLATES 21 AND 22

## INTRODUCTION

The purpose of this paper is to review the types of spirochetes in normal cats, as they are found in the alimentary tract, especially in the mouth, caecum, and large intestine, through study of the fresh material under the dark-field and through the study of fixed stained specimens. A survey of the principal organs including the heart, lungs, liver, spleen, and kidney, is included. The filtration and cultivation of the spirochetes and their implantation in young cats are also dealt with.

Summarizing the historical data, it will be noted that most of the work done on the spirochetes of the cat has involved those of the stomach. Salomon's (16) work deals with the three gastric types. Though Escherich (5) mentioned the fact that spirochetes were found in the caecum and large intestine, he described only a coarse corkscrew form. MacFie (10) noted a type found in the large intestine and rectum which was very loosely coiled. Sanarelli (17) observed that spirochetes lived especially in the large intestine. DeMello and Fialho (4) noted and illustrated only one type similar to the *Spirochaeta eurygyrata*, the human intestinal spirochete. Nowhere in the literature is there a complete survey of the spirochetes of the cat. Only the spiral forms found in the stomach and one type found in the large intestine are described. No reference to the types of spirochetes in the mouth was found.

## EXPERIMENTAL WORK

The study of spirochetes in the cat was divided into the three following divisions: 1. Study of the living material under the dark-field, and observation and measurement of fixed stained specimens. 2. Filtra-

<sup>1</sup> From the Department of Bacteriology, School of Hygiene and Public Health, The Johns Hopkins University, Baltimore. The writer wishes to acknowledge the assistance given by Dr. W. W. Ford and Dr. L. B. Lange in this investigation.

tion and cultivation experiments. 3. Implantation experiments in young cats *per os* and *per rectum*. All work was done on apparently healthy cats secured from various sources. Most of the animals were killed immediately, but a few were kept from 2 days to a week in the laboratory until needed. The animals were not fed for approximately 15 hours preceding the examination.

1. *Study of living material under the dark-field and observation and measurement of fixed stained specimens*

The animals were killed in the laboratory either by ether or gas and the entire alimentary tract removed. The oesophagus, stomach, caecum, and large intestine were each removed with uncontaminated instruments and placed in separate Petri dishes. The small intestine was roughly divided into duodenum, jejunum, and ileum and the three parts placed in separate Petri dishes. The oesophagus, stomach, and intestines were each opened with a pair of sterile scissors and the contents washed out with physiological salt solution. Studies were made under the dark-field of the contents, and of the mucosal scrapings emulsified in Ringer's solution.

A. *Living material*

Under the dark-field four distinct types of spirochetes, the spironeme, treponeme, fusi-spiral, and leptospira, were found in the living material.

*Adult cats.* Forty-eight out of fifty adult cats contain spirochetes in the mouth or gastro-intestinal tracts or both. Spironemes, treponemes, fusi-spirals, and in a few cases leptospiras were observed in the mouth, caecum, and large intestine, the spironemes and treponemes usually in large numbers. Fusi-spirals were scarce in the mouth, but abundant in the caecum and large intestine, especially in the caecal scrapings. Leptospiras were never numerous.

a. Mouth: The organisms differed in size and proportions but four common types stood out. The most common was a spironeme (No. 1), approximately  $10\mu$  long and  $0.5\mu$  thick when stained with the Kliewe and Kliewe-Harris stains. It is usually extremely flexible, the spirals in many cases undergoing frequent change. Some individuals of this type were quite sluggish, sometimes remaining motionless for short periods before resuming their characteristic movement. There are 3 to 6 rather shallow, irregular curves and the ends are sharply tapered (Figs. 1 and 2). In quiescent forms the spirals were more regular. This spirochete was classified in the present summary as a spironeme,

because of its great flexibility and the frequent change in the form of the spirals, relatively shallow and few in number, together with a greater bending and lashing than is seen in the typical treponeme. Though most of the spironemes were from  $10\mu$  to  $15\mu$  long, many were  $20\mu$  to  $30\mu$  and some even longer (Figs. 2 and 3). These were evidently in the stage preceding division as shown in Fig. 2. Their thickness was usually  $0.5\mu$ .

A second spironeme (No. 2) often observed in the mouth appeared under the dark-field as a long thick organism with a definite double contour and wide, flat curves. It is from  $10\mu$  to  $30\mu$  long and  $0.75\mu$  to  $1\mu$  thick (Fig. 2). A similar organism, obtained from the human mouth, was described by Noguchi (15).

A third spironeme (No. 3) is that shown in Fig 4b. This organism was present in the mouth of eleven cats, in the caecal scrapings of two cats, and in the colonic scrapings of one cat. This organism is approximately  $20\mu$  to  $25\mu$  in length and has small, regular, primary spirals and usually two secondary curves of 5 to 6 primary spirals each. The latter remain practically fixed, but there appears to be a continuous change in the primary spirals. The rotation of the body with the resulting progression of the light reflex gives the impression of a wave-like motion along the length of the organism. When stained, both the secondary and primary spirals usually become irregular (Figs. 3 and 4). The ends are pointed. It resembles the *Spirochaeta pseudorecurrentis* of Zuelzer (21), a water spirochete probably with a number of subspecies (Fig. 4a).

A fourth spironeme (No. 4) was noted in fresh material from the mouths of 11 cats. It also occurred in the ileal contents of a kitten about 10 weeks old, and in the contents and scrapings of the caecum of an adult cat, where it was clearly observed under the dark-field, and in fixed, stained specimens among the more common spironemes mentioned above. It is a typical spironeme approximately  $10\mu$  long and  $0.3\mu$  to  $0.5\mu$  thick, with 3 to 6 spirals which are fairly constant when the organism is in motion and maintain their form in fixed preparations (Fig. 5). The spiral depth and amplitude is  $2\mu$ . The ends are pointed but no terminal filaments were seen. A small projection appears at the apex of each rounded spiral, clearly visible under the dark-field and in the films stained by the Kliewe-Harris method. The regularity of these projections and their constancy despite rotation of the spirochete suggest that they may represent a thin lateral extension of the body, or a regular series of protrusions too small to distinguish individually, arising

from the convex surface of the spirals along the length of the organism. Such a continuous or intermittent ridge might account for the microscopic picture. One would expect such a ridge to be seen clearly where it would protrude from the apices of the spirals into a vacant background, but to be seen less clearly if at all, between the apices where, as viewed by the observer, it would lie above or below the body of the spirochete. While the projections from the apices of the spirals appear in stained specimens as they do under dark-field illumination, it has not proved possible up to the present to demonstrate a hypothetical ridge along the body between the spiral apices by any of the staining methods used in this work. A thorough search of the available literature has not disclosed a record of this morphological type. The name *Spironema langei* n. sp., is suggested for this spirochete.

The small lateral protrusions of this spironeme are different from the "knospes" or buds of Meirowsky (12), Fig. 6, in that they appear regularly at the apex of each spiral turn and are delicate and pointed while the "knospes" occur irregularly on the end and laterally near the middle of the spirochetes, and are rounded. Meirowsky gives a good survey on these "knospes." They were considered by some to be a propagation phenomenon and by others a degeneration process. In Fig. 7 an example of the "knospes" is shown on a spironeme from the mouth of the cat seen in the course of this work, which is similar to No. 53 of Fig. 6, Meirowsky's sketches illustrating the "knospes."

As contrasted with the spironemes which were definitely of distinct morphological types, the treponeme group was made up of organisms of various sizes which seemed to grade into one another. The treponemes of the mouth varied in length from  $5\mu$  to  $15\mu$ , exceptionally  $15\mu$  to  $20\mu$ . Most of them averaged  $5\mu$  to  $10\mu$ , the greatest number being  $5\mu$  long. A few long individuals were evidently about to divide. The more numerous type (A) was  $5\mu$  long and  $0.25\mu$  thick, and the less frequent type (B)  $10\mu$  long and  $0.25\mu$  thick, that is, twice the length but the same diameter (Fig. 8). These measurements and the fact that incurvation forms (Fig. 9) were common among the longer individuals suggest that they constitute one species undergoing rapid transverse divisions. A few organisms were somewhat thicker (Fig. 10) and appeared to be a distinct species. The observations did not permit further conclusions as to the number of species represented by these forms. All the principal types of treponemes were found in the mouth.

Leptospiras were found in a few cases in mouth material. They were approximately  $10\mu$  long, very slender, and had extremely flexible hooked



ends. They were noted only occasionally, were few in number, very active, and not always easily detected in a field containing numerous spironemes and treponemes.

b. Esophagus: Though spirochetes of various types were found in great numbers in the mouth, none were observed in the esophagus, although in every animal scrapings emulsified in saline were carefully examined.

c. Stomach: In the gastric contents no spiral organisms were seen, but in the mucosa of 11 cats there was a rigid, coarse organism with closely-set spirals of uniform depth and with abruptly pointed ends. It was not encountered at any other site. In an emulsion of the pyloric mucosa of cat No. 4 a very delicate treponeme resembling *Treponema pallidum* was found. This treponeme appeared more delicate than treponemes A and B mentioned above, both under the dark-field and in fixed stained specimens.

d. Small intestine: The spirochetal flora of the small intestine was scanty. A few spironemes were seen in the duodenal contents of cat 46, the jejunal contents of cats 3, 46, and in the ileal contents of cats 5, 25, 46. Short treponemes, approximately  $5\mu$  long, with a spiral depth of  $0.6\mu$  to  $1\mu$  were found in the ileal contents of cats 13, 44. No spirochetes were observed in the mucosal scrapings of the small intestine.

e. Caecum and large intestine: Treponemes, spironemes, fusi-spirals, and leptospiras were found in the contents and scrapings of the caecum and large intestine. The first three types were found in larger numbers in the mucosal scrapings than in the contents. The spironemes and fusi-spirals were more numerous in the caecal scrapings and the short treponemes were more numerous in the colic scrapings. The majority of the spironemes and treponemes were morphologically similar to the two common forms in the mouth (Spironeme No. 1 and Treponeme A). The longer dividing forms of spironemes and treponemes were not as usual in the caecum and large intestine as in the mouth. Only one spironeme dividing was seen in the caecal scrapings of cat 29. The fusi-spiral was observed in the contents and scrapings of the caecum and large intestine, in greatest numbers in the caecal scrapings. In fixed stained specimens it was approximately  $10\mu$  long and  $0.5\mu$  thick in the middle, tapering gradually toward the sharply pointed ends (Fig. 11), and the spirals were usually irregular. Sometimes they were regular and shallow, one or two in number, with a depth of  $2\mu$  and an amplitude of  $4\mu$ . The movement of this organism is very characteristic. While rotating and advancing it exhibits the typical spiral form

as the reversed motion commences. Sometimes it assumes a circular form with loss of spirals. In our material we also observed, occasionally, the posterior end to become fixed and the anterior end to lash backward forming a circle (Fig. 11). After brief arrest the anterior end would spring forward and the spirals reform as the organism shot forward in the original direction.

As in the mouth, leptospiras were noted only a few times, being found in the mucosal scrapings of the caecum and large intestine of 5 cats. They were approximately  $10\mu$  long, very slender with very active hooked ends. They were seen only in small numbers under the dark-field and in fewer numbers in stained preparations.

Summary of the findings in adult cats according to types of organisms: Spironemes and treponemes are the most common types throughout. The commonest spironemes (No. 1) are approximately  $10\mu$  long and  $0.5\mu$  thick with 3 to 6 irregular spirals, the commonest treponemes (A) are approximately  $5\mu$  long,  $0.25\mu$  to  $0.3\mu$  thick, with 4 to 8 regular close-set spirals. The treponemes and spironemes from the mouth, caecum, and large intestine were indistinguishable morphologically either under the dark-field or in fixed stained specimens. Fusi-spirals, leptospiras, and a coarse rigid spiral organism (stomach) are less common.

Summary of the findings in adult cats according to distribution in the animal body: In the mouth, spironemes, treponemes, fusi-spirals, and leptospiras were found. The most common forms were the spironemes (No. 1) and the treponemes (Type A). In the esophagus no spirochetes were noted. In the mucosal scrapings of the stomach, a few spiral organisms were observed. They were rigid, coarse, with closely-set spirals of uniform depth, and abruptly pointed ends. This type was not seen in any other part of the alimentary tract. In the pyloric wall of one cat a few treponemes (similar to *Treponema pallidum* and more delicate than Types A and B) were demonstrated. Rarely the contents of the small intestine showed a few spironemes and a very few treponemes. Fusi-spirals, spironemes, and treponemes were found in large numbers in both contents and scrapings of the caecum and large intestine. In none of the 84 cats examined were spirochetes found in the lungs, liver, spleen, heart, or kidney.

*Kittens.* The kittens studied ranged in age from mature embryos to approximately 90 days. In the 22 apparently normal kittens examined no spirochetes were found in the alimentary tract of those younger than 60 to 90 days. The type most often noted in older kittens was the spironeme, which was especially numerous in the mucosal scrapings of

the caecum of two kittens. Spirochetes were also found in the large intestine, more numerous in the mucosal scrapings than in the intestinal contents. Treponemes were found in a few cases in the scrapings of the large intestine, usually in moderate numbers but in one case they were very abundant. Treponemes were not observed in the contents of the large intestine. In none of the 22 apparently normal kittens were spirochetes noted in the liver, lungs, spleen, heart, or kidney.

### *B. Stained material*

In staining the organisms, it was found relatively easy to stain both the spirochetes from the mouth and those from the caecum and large intestine by many different methods including Giemsa, Fontana-Tribondeau (20), Kliewe (9), Kliewe-Harris (6), Romanowsky (8), Medalia's modification of Wright's stain (11), diluted carbol fuchsin (16), Loeffler's methylene blue (18), gentian violet (19), Cross' stain (3), Benian's "relief stain" (1), and Burri's method (2). Spirochetes from cultures were somewhat more difficult to stain. Fontana-Tribondeau, Kliewe, and Kliewe-Harris were found most satisfactory and were used regularly. Various modifications of these stains were tried from time to time but no noticeable improvement over the original methods was observed.

In the examination of fixed stained material, only 2 types were recognized, the treponemes and spirochetes. The fusi-spirals appeared similar to the spirochetes when stained. They were usually S-shaped and contained one complete shallow spiral.

The treponemes and spirochetes found in the mouth, caecum, and large intestine were indistinguishable morphologically, either under the dark-field or in fixed stained specimens.

## *2. Filtration and cultivation experiments*

### *A. Filtration*

Fourteen out of 18 filtrations by gravity and all of the filtrations with pressure of 6 pounds yielded spirochetes. The gravity filtrations were centrifuged at low speed for 5 to 10 minutes, the supernatant fluid decanted and the few remaining drops used. The spirochete was the only type of spirochete seen in the filtrates. In several filtrations with the Berkefeld N candle the spirochetes alone came through, no bacteria appearing in the dark-field specimen. In the filtrations with the Berkefeld V under pressure a few bacteria were seen. When the filtrates were cultured as described below all contained bacterial contaminations. Filtered spirochetes were used in the cultivation experiments. When

filtrations were done with pressure the first few drops of the filtrate were used for cultivation and only after preliminary dark-field examination to exclude so far as possible the presence of bacteria.

### *B. Cultivation*

Summarizing the results of approximately 600 tubes of fluid and semi-solid media inoculated with mouth, caecal, or intestinal material, 138 tubes were found upon examination to show greater or less increase of the spirochetes. All successful cultures were grown aerobically at 37°C. in media with a pH of 7.0.

Good growth of caecal and intestinal strains was obtained in Noguchi's diluted fluid medium (13) containing fresh cat liver and autoclaved rabbit liver, 3 successive subcultures being made from tubes of this type. The spirochetes were never obtained in pure cultures.

The mouth spirochetes did not multiply in this medium. A mixture of 5 cc. ascitic fluid and 5 cc. distilled water was found to give the greatest increase in number of mouth and intestinal spirochetes in the shortest time (approximately 2 days). Successful subcultures were also made in this medium. Hogue's ovomucoid medium (7) yielded growth of spirochetes from the mouth, caecum, and large intestine. Subcultures were also obtained in these cases. Noguchi's soft, semi-solid medium (14) was especially good for growing spirochetes from the mouth of the cat.

### *5. Implantation experiments in young cats per os and per rectum*

Young cats 3 to 15 days old were used for the implantation experiments, because preliminary examination had shown that animals younger than 2 months did not have spirochetes in the mouth or gastrointestinal tract. Five groups of animals were used, lots of 3, 4, 3, 3, and 4 kittens constituting the 5 groups which were 13, 14, 15, 4, and 3 days old respectively when the experiments began.

Introduction of spirochetal material extended over a period of 2 weeks. The first 3 groups were fed the emulsified scrapings of the large intestine containing spirochetes of all types. The fourth group was fed the emulsified mucosal scrapings and diluted caecal contents of a hooded rat containing spirochetes morphologically similar to those found in the caecum and large intestine of the cat. In the fifth group half were given mouth spirochetes *per os* and half *per rectum*.

Summarizing the results, both mouth and intestinal types were implanted in the caecum after feeding *per os*. Mouth types were also

implanted in the caecum after introduction *per rectum*. A heavier implantation was secured when a preliminary dose of saturated solution of sodium sulfate had been administered. The mouth, liver, lung, heart, kidney, and spleen contained no spirochetes.

#### SUMMARY AND CONCLUSIONS

1. Forty-eight out of 50 normal adult cats contained spirochetes in the mouth or gastro-intestinal tract or both. Five out of 22 normal kittens, ranging in age from mature embryos to approximately 90 days, contained spirochetes in the caecum and large intestine. The animals containing these organisms were 70-90 days old.

2. The usual types of spirochetes observed were spironemes, treponemes, and fusi-spirals, present in large numbers in the alimentary tract. The first two types were abundant in the mouth, caecum, and large intestine. The fusi-spirals, while present in the mouth and large intestine, were most abundant in the caecum. Spironemes or treponemes or both were occasionally encountered in the duodenum, jejunum, or ileum. Spiral organisms with rigid, coarse, blunt spirals were found in a few stomachs. Leptospiras in relatively small numbers were also present at times in material from the mouth, caecum, and large intestine.

3. The most common type of spironeme in mouth or intestinal tract was approximately  $10\mu$  long and  $0.5\mu$  thick having 4 or more spirals about the same depth in the middle as at the ends with a depth of  $0.6\mu$  to  $1.0\mu$  and an amplitude of  $1.0\mu$ . A spironeme was also noted in material from the mouth of the cat which we regard as a new species and for which we suggest the name *Spironema langei*. It is a typical spironeme approximately  $10\mu$  long and  $0.3\mu$  to  $0.5\mu$  thick, with 3 to 6 spirals which were fairly constant when the organism was in motion and maintained their form in fixed preparations. The spiral depth and amplitude was  $2\mu$ . The ends were pointed and no terminal filaments were seen. A small projection occurred at the apex of each rounded spiral, clearly visible under the dark-field and in the films stained by the Klieve-Harris method (Fig. 5). The fusi-spirals were  $6\mu$  to  $10\mu$  long,  $0.5\mu$  to  $0.75\mu$  thick in the center and tapering toward the ends which were sharply pointed. The organism when stained was S-shaped, usually contained one complete shallow spiral. The leptospiras were approximately  $10\mu$  long, very slender, with closely-set spirals and very flexible hooked ends. These types occurring in the mouth, caecum, and large intestine were morphologically indistinguishable.

4. The heart, lungs, liver, spleen, and kidney of 86 apparently normal cats and kittens did not contain spirochetes.

5. Cats younger than 2 months did not show spirochetes in the mouth or gastro-intestinal tract. Under natural conditions the spirochete was the first to appear in young cats.

6. All the organisms were satisfactorily stained. The Fontana, Kliewe, and Kliewe-Harris methods were the most satisfactory. Very delicate treponemes staining with more difficulty than *Treponema pallidum* were well demonstrated by the Kliewe-Harris method.

7. Spirochetes from the caecum and large intestine were filtered through Berkefeld N and V candles by gravity in 5 to 6 hours, and by a pressure of 6 pounds in 5 to 10 minutes. The filtrate from the Berkefeld N candles was apparently free from bacteria when examined under the dark-field.

8. Growth was obtained in various media though the spirochetes were never obtained in pure culture. Successful cultivation of spirochetes from the mouth, caecum, and large intestine through two subcultures was obtained in 5 cc. ascitic fluid diluted with an equal amount of distilled water, both raw and filtered material being used. Successful cultivation with 3 subcultures was also obtained with diluted ascitic fluid (1:3), containing a piece of autoclaved rabbit liver, and in Hogue's ovomucoid medium. They were successfully grown in Noguchi's fluid medium but did not grow in the diluted ascitic fluid with liver tissue. All successful cultures were incubated aerobically at 37°.

9. Spirochetes were successfully implanted in kittens 5 to 15 days old, mouth spirochetes of the adult cat being implanted *per os* and *per rectum* and those from the large intestine *per os*.

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CHARLOTTE, N. C.

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## EXPLANATION OF PLATES

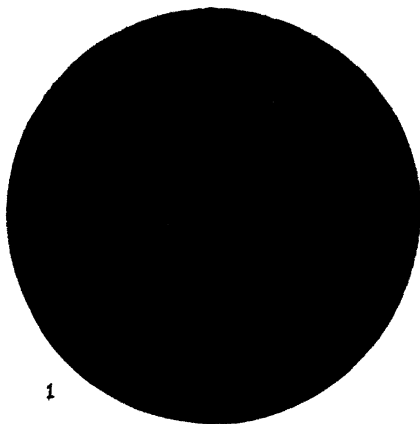
## PLATE 21

- Fig. 1. Spironeme No. 1. Short and longer forms. Thicker forms, Spironeme No. 2, also present. Source, mouth. Photomicrograph  $\times 900$ .
- Fig. 2. Spironemes No. 1 and No. 2. Spironeme (lower right) in process of transverse division. Source, mouth.  $\times 900$ .
- Fig. 3. Spironeme No. 2. Short and longer forms. Spironemes No. 3 (center) faintly stained. Source, mouth.  $\times 900$ .
- Fig. 4. (a) Zuelzer's *Spirochaeta pseudorecurrentis*. Handbuch der Pathogenen Protozoen 11: 1670. 1925.  $\times 900$ .  
(b) Spironeme No. 3. Spirochete from mouth of cat similar to Fig. 4a, in living material—spirals irregular when stained.  $\times 900$ .
- Fig. 5. Spironeme No. 4. *Spirocheta langei*, diagrammatic representation. Source, mouth.
- Fig. 6. Knospen. Meirrowsky, Med. Klin. 12: 1181. 1916. Photomicrograph.  $\times 900$ .

## PLATE 22

(Photomicrographs  $\times 900$ )

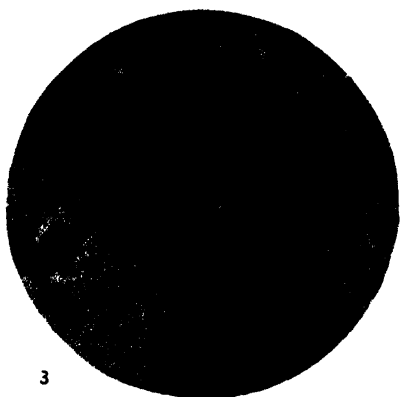
- Fig. 7. Knospen on Spironeme from mouth of cat, similar to No. 53 of Fig. 6.
- Fig. 8. Treponemes A and B, short and longer forms. Source, mouth. Spirochete No. 1.
- Fig. 9. Treponemes A and B. Thicker forms of treponemes showing incurvation.
- Fig. 10. Thicker forms of treponemes.
- Fig. 11. Fusi-spirals.



1



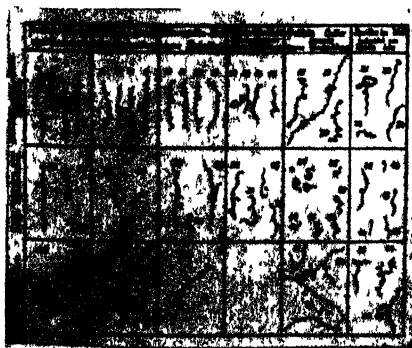
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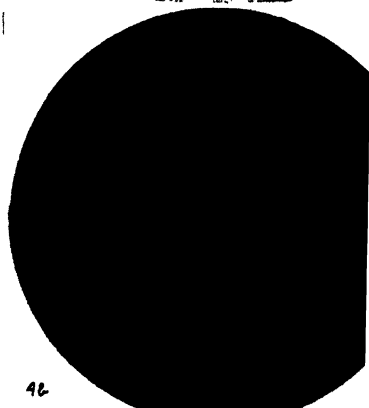
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4a

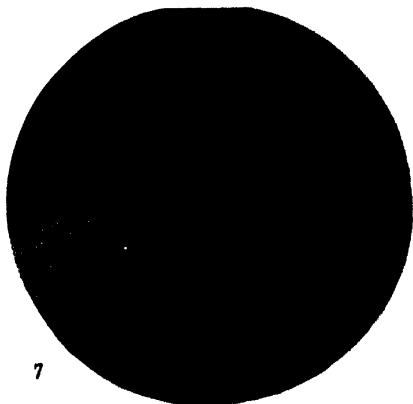


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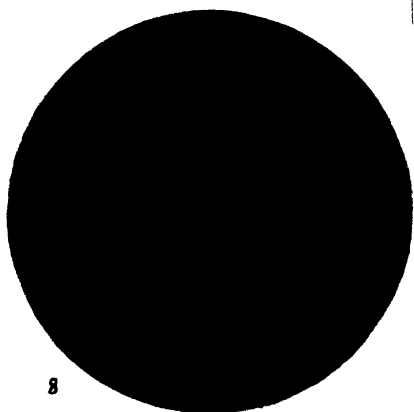




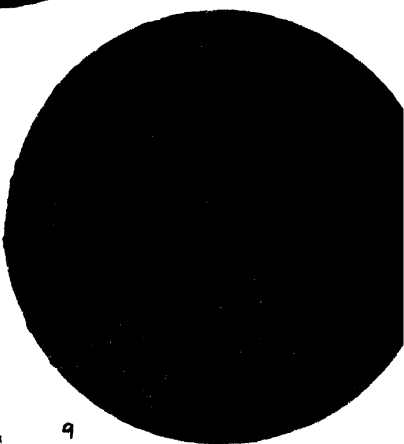




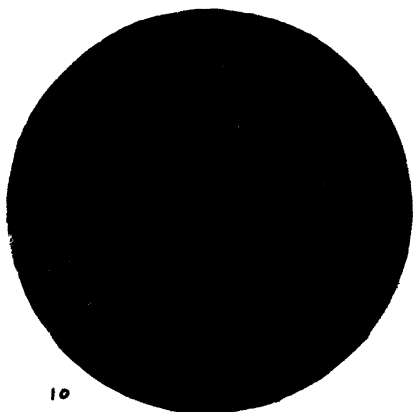
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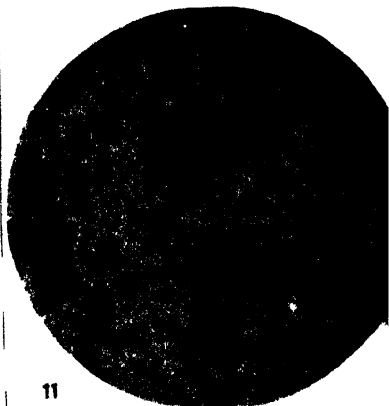
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11



# THE LIFE HISTORY OF POLYPODIUM POLYPODIOIDES HITCH., ESPECIALLY SPERMATOGENESIS<sup>1</sup>

By DOUGLASS E. RANKIN

PLATES 23-26

## INTRODUCTION

The following study of the gametophyte of *Polypodium polypodioides* was carried out in search of a better understanding of the behavior of the nucleus, the plastids, and mitochondria during the several phases of development of this fern.

This work includes a general study of the development of the antheridium, of the archegonium, of fertilization, and of the development of the embryo. A cytological study of spermatogenesis, carried on not only with nuclear fixatives but also with cytoplasmic and mitochondrial ones, is likewise included.

## HISTORICAL SURVEY

The structure and development of the antheridium and archegonium of the Leptosporangiate ferns have been extensively studied. A rather complete bibliography of the subject up to 1923 has been published by Bower (7) in his book on the Filicales and need not be repeated here. More recent work on these phases in certain Leptosporangiatæ has been done by Miss Stokey (31) on the Cyathaceæ and by Miss Hartman (16) on the opening of antheridia in certain Polypodiaceæ.

Comparatively few workers have studied fertilization in the ferns. Studies have been published by Strasburger (32) on *Pteris* and *Ceratopteris*, by Campbell on *Pilularia* (9) and on *Osmunda* (10), by Shaw (29) on *Onoclea*, by Thom (34) on *Adiantum* and *Aspidium*, and by Yamanouchi (37) on *Nephrodium*.

As mentioned by Yamanouchi (37), the two features of especial interest recorded in the above papers are:

1. The presence of a wall around the egg after fertilization.
  2. The frequently collapsed condition of the egg after fertilization.
- Strasburger (32) has stated that more than one sperm is prevented

<sup>1</sup> Botanical Contribution from the Johns Hopkins University No. 126.

from entering the egg by the formation of a heavy enclosing wall about this immediately after fertilization by the first one. Shaw (29) on the other hand believes that a partial collapse of the egg cell occurs after one sperm has entered. In this condition additional sperms are not able to enter. Mottier (20) doubts that the above condition is normal.

The development of the embryo of the Leptosporangiateae has been studied by Atkinson (2), Campbell (10), Leitgeb (19), Shaw (29) and, more recently, by Cross (11). The occasional formation of more than one embryo on a prothallus was noted by Atkinson (2), Etter (14), and Mottier (22).

Strasburger (32 and 33), Belajeff (3, 4, 5, 6), and Shaw (30) made important studies of the spermatogenesis of ferns during the latter part of the nineteenth century. A question under debate with most of these workers was whether or not the blepharoplast is homologous with the centrosome. Strasburger (33), from his work on the zoospores and gametes of the algae and on the spermatozooids of ferns, concluded that the cilia on both arise from the blepharoplast and that these structures are morphologically distinct from centrosomes. Belajeff (5) was the earliest worker to describe the development of the blepharoplast in Pteridophytes. He first saw the cilia arising from the blepharoplast in the spermatozooids of *Equisetum*. The work of Belajeff (5) and Shaw (30) on the spermatogenesis of *Marsilea* led both to conclude that the blepharoplasts in that plant are identical with centrosomes. Webber (35) and Ikeno (18) worked on the spermatogenesis of the Cycadaceae. Webber believed the cilium-bearing organ to be quite distinct from the centrosome and it was he who named the former the "blepharoplast." Ikeno, however, believed the two organs to be identical.

In discussions of the morphological development of plant spermatozooids, the interest until recently has been centered in the metamorphosis of the nucleus and of the cilium-bearing organ. Of late, however, there has become evident an interest in certain cytoplasmic elements other than the blepharoplast; namely, the chondriosomes, the plastids, and the vacuoles. The part that these structures play in spermatogenesis is still a matter of uncertainty and of controversy.

Yamanouchi (37), in his study of the spermatogenesis of *Nephrodium molle*, found that the blepharoplasts are first discoverable at the resting stage before the last nuclear division in the antheridium. At this time they appear as two darkly staining bodies, one on either side of the nucleus. They remain near the pole of the spindle during the division but no asters are found around them. When the spermatids are formed

one of these bodies is seen in each cell some distance from the nucleus. At the same time there appears opposite the blepharoplast a smaller body which is called the Nebenkern. The blepharoplast applies itself to the nuclear membrane and elongates along with the nucleus, following half way around the two and a half times coiled spermatozoid. The Nebenkern remains in the cytoplasm at the side of the nucleus and is finally found in the vesicle attached to the lower end of the spermatozoid.

Certain of these same structures were found by R. F. Allen (1) in her work on spermatogenesis in ferns. She did not, however, discover blepharoplasts in the spermatid mother cell nor find a structure comparable to the Nebenkern. On the other hand she did, like Buller (8), find starch in the vesicle of the spermatozoid, a fact which earlier workers had noted but which more recent investigators had not mentioned.

In *Equisetum*, Sharp (27) found a single centrosome surrounded by astral radiations in the penultimate generation of the antheridium. This divides to form the blepharoplasts which pass, with their asters, to opposite sides of the nucleus and occupy the poles of the spindle. In the spermatid the blepharoplast becomes vacuolated and fragments. Later these parts unite to form the cilium-bearing band. This band becomes associated with the nucleus in forming the spermatozoid but is not so closely bound to it as is the case in the ferns. He also found a series of darkly-staining bodies along the concave surface of the nucleus, the origin or nature of which he did not determine.

In a study of the spermatogenesis of *Marsilea* also, Sharp (28) decided that the blepharoplast is of the nature of a centrosome. In the first mitosis of the spermatogenous tissue the two centrioles are weakly developed but show astral radiations. In the second, third, and fourth divisions, however, they are very distinct. The blepharoplast becomes vacuolate and fragments as in *Equisetum*, but to a less degree. The parts here also later unite to give rise to the band which produces the cilia.

So little work has been done in all plants on the cytoplasmic elements other than the blepharoplast that the discussion of the literature on these subjects will be more general in nature.

Gavaudan and Cazalis (15) studied the behavior of the plastids in the Characeae during spermatogenesis. They found that the plastids retain their normal appearance until the spermatid begins its transformation into the spermatozoid. The plastids are then said to form a granular band on the posterior surface of the spermatozoid.

Motte (20), from his study of spermatogenesis in the mosses, con-

cluded that both mitochondria and plastids are present in the early stages of the development of the antheridium. In the later stages of spermatogenesis the plastids lose their starch and fragment. These fragmented plastids plus the mitochondria condense to form the solid limosphere which is attached to the posterior portion of the spermatozoid nucleus.

Weier (36) traced the development of the plastid through spermatogenesis in *Polytrichum* and *Catharinaea*. The young antheridia of these mosses, according to Weier, possess many plastids but their number becomes reduced by successive cell divisions until each androgone has only one plastid. This divides prior to each of the next two divisions. During the last division the plastid becomes reticulate. In the androcyte this reticulate body condenses to form the limosphere. The limosphere buds off the apical body and the remainder forms the vesicle at the base of the sperm. The apical body comes in contact with the blepharoplast which draws it to the anterior part of the sperm where it forms a slender pointed cytoplasmic filament.

In his work on the origin and evolution of the plastids in Pteridophytes, Emberger (13) discusses the spermatogenesis of *Adiantum*. He finds plastids and mitochondria present in the antheridium initials. The plastids become smaller and finally in the mother cell of the spermatid are hardly distinguishable from the mitochondria. Vacuoles filled by phenolic compounds are found in the early stages of the antheridium and are always present in protoplasts of the wall cells. These disappear by the time the spermatid mother cells are formed. In the spermatids there appear vacuoles, the nature of the contents of which was not determined. The granules at this time are indistinguishable except for a slight difference in size. There is, however, as Emberger thinks, a separation of the granules in the subsequent development of the spermatozoid. The plastids migrate to the vesicle and the mitochondria form a double row of granules along the length of the spermatozoid.

Dracinschi (12) studied the mature sperms in several Eufilicneae, and in *Pilularia* of the Hydropteridineae. She discusses the structure of the sperm under the headings of the cilium-bearing band, the nuclear substance, and the plasma material. The cilium-bearing band and nucleus form the spiral body of the sperm. The cilium-bearing band is broad at the anterior end and tapers toward its base. It is somewhat more than half the length of the spermatozoid. It bears three rows of cilia on the anterior end and the number of rows decreases to one as the band becomes smaller. Each cilium possesses a small, expanded, basal

portion and a stiff shaft about two microns in length. The remainder of the cilium is flexible and fibre-like in character. A darkly-stained zone which she calls the *Randsaum* (or marginal hem) runs along the outer anterior edge of the sperm. This, according to Dracinschi, is the structure that Belajeff (5) thought gave rise to the cilia and is what Shaw (30) later mistakenly called the blepharoplast. The nucleus is covered with a thin cytoplasmic sheath. In this sheath, on the outer, posterior surface of the spermatozoid, are found plate-like bodies which Dracinschi thought to be Golgi material. Chromomeres are found in the nucleus. Adhering to the inner margins of the coils of the nucleus is a row of granules, the morphological nature of which is not known but which Dracinschi thought might be of either plastid or vacuolar nature.

Yuasa (38) also studied the mature sperms of *Leptosporangiate* ferns. He found that in *Pteris cretica* the cilium-bearing band and the marginal hem both run the whole length of the sperm.

#### MATERIALS AND METHODS

The prothallia used in the following experiments were obtained from spores of *Polypodium polypodioides* collected near Fayetteville, North Carolina. The spores were sown on powdered peat which had been sifted and boiled. The spores germinated in about ten days. Antheridia were formed after eight weeks and archegonia in approximately ten weeks.

Bouin's fixation was employed for fixing the prothallia used in the morphological study of the development of the sexual organs, of fertilization, and of the development of the embryo. The two most successful stains employed were Heidenhain's iron haematoxylin for the chromatin of the nuclei and Delafield's haematoxylin for the cell walls.

For the work on spermatogenesis various other fixing agents and stains were employed to bring out the different cell components. Flemming's weaker fixative followed by Heidenhain's haematoxylin was used for the blepharoplast and nucleus. Prothallia fixed in Bouin's mixture, after pre-fixation with ten per cent formalin neutralized with  $MgCO_3$ , gave striking pictures of the blepharoplast and also of the development and the various phases of structure of the nucleolus. The chromosomes were fixed by this technique, but the chromatin reticulum was not stained. Champy-Kull's fixative and Altmann's stain fixed and stained mitochondria, plastids, blepharoplasts and nucleoli. By this method the blepharoplast and nucleus of the mature spermatozoid were differ-



entiated; other combinations failed to show this. The mitochondria when fixed with Regaud's medium and stained with Heidenhain's haematoxylin were seen more distinctly than by any other method. The mitochondria in the mature spermatozoid were especially clear. Starch in the mature spermatozoid stained purple with gentian violet after Merkel's fixative. It was also stained in the spermatid and spermatozoid with Gram's iodine stain, following fixation by Regaud's method. Several other fixatives and stains were used but gave no essentially different results and hence will not be discussed here. Mann-Kopsch's fixing liquid showed the development of plastids; Bouin's fixative, with Feulgen's reaction as a stain, was used for the chromatin. Vital stains such as neutral red, methylene blue, and Janus green were employed also, but without satisfactory results.

MORPHOLOGICAL STUDY OF THE REPRODUCTIVE ORGANS, FERTILIZATION,  
AND THE DEVELOPMENT OF THE EMBRYO

*Development of the prothallus.* The first sex organs to appear on prothallia of *Polypodium polypodioides* are the antheridia. These occur on plants which are about two months old and are formed before the prothallium has become heart-shaped, or at least soon after the notch appears. The antheridium-bearing plants are small and one or more cells in thickness (figs. 1, 2). In some cases the antheridia cover practically the entire upper and lower surfaces of the prothallium, leaving only a narrow margin of sterile vegetative cells across the apical end. Such plants apparently never form archegonia. Other male plants which have not borne such a heavy crop of antheridia continue vegetative growth for some time and then form archegonia.

The first archegonia were formed on prothallia of the same sowing as that last mentioned which had *not* borne antheridia. They developed about two weeks after antheridia had appeared. The female plants were more characteristically heart-shaped and about three times as large as the male plants of the same culture. They possessed the typical archegonial cushion along the central portion of the prothallium. The archegonia are formed on this cushion from both the upper and lower surfaces. The archegonia form on the lower surface first, as is also true of antheridia. Only rarely were both types of reproductive organs found on the same plant at one time. When this did occur the antheridia were formed on proliferations from the margins or cushions of plants the bodies of which had previously borne no antheridia but archegonia only. A culture of *Polypodium polypodioides* about four months

old is shown in figure 3. This photograph shows the archegonial plants distinctly but the smaller, antheridial prothallia are not clear.

*The development of the antheridium.* The development of this organ resembles that found in many other Polypodiaceae. A prothallial cell enlarges and divides at an often slightly oblique angle, to give rise to a basal cell and an antheridium initial (fig. 42). The first division in the initial produces a cup-shaped cell whose rim touches the lateral wall of the antheridium initial and whose base may or may not come into contact with the upper wall of the basal cell (fig. 22). The second wall is concentric with the upper surface of the antheridium and intersects the first wall in a ring (fig. 22). In this upper cell is formed the third wall, a circular anticline, which cuts out a disk-like cover cell with sloping sides (fig. 23). It is this latter cell which, by bursting, or by being dislodged intact, allows the spermatozoids to escape. The three cells so formed surround a large central cell, which is the primary spermatogenous cell. In the central cell the first division is longitudinal and the second one transverse to the axis of the antheridium. After this one, two, or rarely three more divisions may occur, giving respectively 8, 16, and 32 spermatids. In some of the smaller prothallia none of the antheridia had more than eight spermatozoids. Generally, however, about half of the antheridia of the smaller plants produced eight spermatozoids each and half produced sixteen spermatozoids. On the larger prothallia, on the other hand, about one-third of the antheridia produced eight spermatozoids, and two-thirds produced sixteen. Sometimes a slightly larger proportion of the antheridia produced sixteen spermatozoids each, i.e. larger prothallia have larger antheridia. Antheridia with thirty-two spermatozoids were rare.

The mode of opening of the antheridium corresponds with that described by Miss Hartman for certain other Polypodiaceae. The cover cell, though it is occasionally extruded intact, is more often burst and its contents form a granular mass.

*Development of the archegonium.* The archegonia arise in acropetal succession on the cushion region of the prothallium. The initial cell of the archegonium is formed by a division of a superficial cell in this region (fig. 4). It can be identified by its large nucleus and by its cytoplasm which is denser than that of the surrounding vegetative cells.

When the archegonium initial is formed it undergoes two successive transverse divisions, giving rise to the basal cell, the central cell, and the primary neck cell (fig. 5). The central cell is, like the archegonium initial, conspicuous because of its large nucleus and dense cytoplasm.

The first division in the young archegonium is in the primary cover cell (fig. 6). The cell wall so formed is an anticline parallel to the axis of the archegonium. The two neck cells immediately undergo another anticlinal division perpendicular to the first. Thus are formed the four neck cells that are present at this time in the archegonium. The central cell, meanwhile, has started to enlarge. As it increases in size the neck cells divide by anticlines transverse to the neck and so keep pace with it, and in this manner is formed a neck which projects beyond the surface of the prothallus.

The first division in the central cell is transverse (fig. 7). This division gives rise to a large inner cell which is embedded in the vegetative tissue of the prothallus and a smaller primary neck canal cell that is surrounded by the wall cells of the neck which project beyond the prothallus. The inner or ventral cell divides before the primary neck cell. When the spindle for this division is formed it is situated in the outer portion of the cell (fig. 8). Thus when the wall is formed the two resulting cells are of quite unequal sizes. The larger inner one is the egg and the smaller outer one is the ventral canal cell. Following this division in the ventral cell a nuclear division takes place in the primary neck canal cell. This is also a transverse division. Most often a cleavage of the cytoplasm occurs, but no wall is formed between the two nuclei which are produced by this division (fig. 10). In some few cases, however, a wall may occur.

Thus the mature archegonium contains a large egg cell, a smaller ventral canal cell, and two neck canal cells or nuclei. As the archegonium matures the egg cell becomes indented due to pressure of the three canal cells above it (fig. 9). Then too, as the archegonium becomes older the two nuclei of the neck migrate to the outer end of the neck and become packed tightly together giving them, superficially, the appearance of being fused.

When prothallia bearing these mature archegonia are watered, the cells of the neck become more turgid and the inner cells swell. As a result the archegonium is ruptured at the apex. This leaves a chimney-like neck with an opening above, through which a slimy substance is discharged. The slimy substance is evidently a disintegration product of the three canal cells. The egg cell which was indented before the opening of the archegonium becomes rounded when pressure is released by the discharge of part of the contents of the neck.

*Fertilization.* The spermatozoids are attracted to the neck of the open archegonium. Pfeffer (26) and Hoyt (17) have noted that this

is due to the presence of malic acid or malates in the disorganized contents of the ventral canal and neck canal cells. Many spermatozoids were observed in the neck of the archegonium but never more than one could be found to have penetrated the egg (fig. 12). After fertilization eggs often presented a collapsed appearance, though other eggs, also containing spermatozoids and in approximately the same stage of development, were well rounded. No unusually heavy wall was observed around the recently fertilized eggs. The spermatozoid while in the neck of the archegonium is very much elongated and loosely coiled (fig. 11). When, however, it has entered the egg the spermatozoid condenses to form a thick, coiled body (fig. 12). The chromatin of the egg nucleus during the penetration of the sperm forms a coarse net. The sperm nucleus next becomes reticulate (fig. 13). Soon the reticulum of the male nucleus becomes indistinguishable from the reticulum of the egg nucleus.

*Development of the embryo.* The fertilized egg enlarges greatly during the resting stage preceding the first division (fig. 14). The first wall formed is slightly oblique to the neck of the archegonium and transverse to the long axis of the prothallus (fig. 15). This divides the embryo into anterior and posterior halves. The second wall is transverse to the neck of the archegonium and parallel to the long axis of the prothallus (fig. 16). The embryo is thus divided into quadrants. The next wall is the octant wall which is parallel to the neck of the archegonium and in the plane of the long axis of the prothallus. Two each of these octants are used in the formation of the primary organs of the plant. In an embryo developed on the lower surface of the prothallus the cotyledon is formed in the lower anterior segments, the stem from an upper anterior segment, the root from a lower posterior segment and the foot from the upper posterior segments. The walls developed within the octants are somewhat irregular. The first walls, however, are nearly always parallel to the anticlinal octant walls (figs. 17 and 18).

The initials are distinguishable even in embryos as young as that shown in figure 18. An older stage showing the root, stem, and cotyledon initials is illustrated in figure 19. In this embryo the quadrant walls are also distinct. A still older embryo in which the root and cotyledon have elongated is shown in figure 21.

CYTOLOGICAL STUDY OF SPERMATOGENESIS—STRUCTURE OF THE SPERMATID AND THE SPERMATOZOID AS DETERMINED BY THE USE OF SEVERAL CYTOLOGICAL METHODS

1. *Results with Flemming's fixative and Heidenhain's haematoxylin stain*

In material fixed and stained by this combination of methods the chromatin and the blepharoplasts are dark blue. This is an excellent fixative and stain for these elements and their development can easily be demonstrated with it. The central cell has a large nucleus and one or two large and often irregular nucleoli (fig. 22). With this technique the nucleoli are distinguishable up to, and sometimes after, the development of the spermatids but never show as distinctly as with the two methods to be described next.

The blepharoplasts are first discoverable in the resting stage of the spermatid mother cell (fig. 24). They appear as two darkly staining bodies, one lying on each side of the nucleus at some distance away from the wall of the latter. The chromatin of the nucleus then forms a coarse reticulum with distinctly thicker and thinner portions. At prophase the blepharoplasts move still farther away from the nucleus and occupy the poles of the spindle, but show no astral radiations (fig. 25). The metaphase, telophase, and anaphase were not found in cells fixed and stained by this method. Cells fixed by Champy-Kull's method showed the blepharoplast during the metaphase. During the resting stage of the spermatid nucleus the blepharoplast appears as a very conspicuous black body lying in the cytoplasm at some distance from the nucleus and the chromatin of the latter appears as a dense network (fig. 26). The blepharoplast approaches the nucleus and finally becomes applied to the nuclear membrane (fig. 27). At this stage it begins to elongate (fig. 28). When the blepharoplast has extended a little over half way around the still globular nucleus, the latter begins to change its form and inner structure. The threads of the chromatin network become more and more thickened (fig. 29) while the side of the nucleus opposite the blepharoplast becomes at first flattened and finally concave (fig. 30). During the time the nucleus is thus crescent shaped in lateral view the blepharoplast *cannot be differentiated with haematoxylin*. When the chromatin has condensed to form the more attenuate, coiled spermatozoid nucleus, however, the blepharoplast can be shown, extending approximately one coil beyond the nucleus, which itself shows from one and one-fourth to one and one-half turns (fig. 31).

*2. Results obtained with neutral formalin, Bouin's fixative, and Heidenhain's haematoxylin stain*

This method fixes and stains nucleoli, blepharoplasts, and the chromosomes. It does not stain chromatin when in the reticulum stage. The central cell shows one or two large, and often irregular, nucleoli (fig. 33). In the two-celled stage some cells were found with one nucleolus, some with two nucleoli and some with nucleoli in the process of division by constriction. Nucleoli in one or the other of these three phases were found in the resting nuclei of all stages up to the time the spermatid nucleus began its metamorphosis into a spermatozoid. The following tables show the combinations of those cell stages and nucleolar phases that were actually found:

- (1) Four-celled stage.
  - (a) Each of the four cells of the antheridium contained one nucleolus.
  - (b) Each of the four cells of the antheridium contained two nucleoli.
  - (c) Three cells of the antheridium contained two nucleoli and one had a dividing nucleolus.
- (2) Eight-celled stage.
  - (a) Each of the eight cells of the antheridium contained one nucleolus.
  - (b) Five of the eight cells of the antheridium contained two nucleoli and three cells contained one nucleolus each.
  - (c) One cell of the antheridium contained two nucleoli and seven cells contained one nucleolus each.
- (3) Sixteen-celled stage.
  - (a) Each of the sixteen cells of the antheridium contained one nucleolus only.
  - (b) Each of the sixteen cells of the antheridium contained two nucleoli.

This technique makes the blepharoplasts even more conspicuous than does Flemming's, and the same developmental stages of these were found.

*3. Results with Champy-Kull's fixative and Altmann's stain*

These fix and stain blepharoplasts, mitochondria, plastids, and nucleoli distinctly, but stain chromosomes only faintly. In addition to the nucleus and the plastids this method demonstrates the presence in the prothallial cells of certain small purple-staining granules. In the prothallial cell at the mitotic division preceding the formation of the antheridium initial the mitochondrial granules, which have increased in number, are grouped about the poles of the spindle (fig. 42). Thus, when the initial of the antheridium is formed, it contains the plastids

which were in the outer part of the prothallial cell and the mitochondrial granules which surrounded the daughter nucleus. In the wall cells there are numerous plastids, smaller in size than those of the prothallial cells. The mitochondria in the wall cells are more numerous than in the prothallial cells but not as abundant as in the spermatogenous cells. These latter cells, on the contrary, though they have many more mitochondria, have fewer and smaller plastids than the wall cells (fig. 43). The plastids in both can be recognized by their deeply-staining outer portion and lightly-staining central region. The cells resulting from the divisions up to the spermatid mother cell stage are essentially alike. The mitochondria are very numerous and grouped about the poles during mitosis. The plastids up to this spermatid mother cell stage are still easily distinguishable. The one or two nucleoli of each cell stain faintly.

With this technique the blepharoplasts appear at metaphase of the division of the spermatid mother cell, as two conspicuous red bodies in each cell (fig. 46). The spermatid at its formation contains numerous mitochondria scattered through the cell (fig. 47). It also contains a large blepharoplast located at some distance from the nuclear membrane. The development of the blepharoplast has been described above. With other techniques, however, it was not distinguishable in the mature spermatozoid. With Champy-Kull's method the red stained blepharoplast can be seen to extend beyond the purple stained nucleus and is applied to a relatively small part of the upper portion of the nucleus (figs. 50, 51). As the nucleus begins to coil, preparatory to forming the spermatozoid, the mitochondria seem to become more closely associated with it (fig. 49). In the mature spermatozoid they form a double row of granules on the inner surface of the coiled nucleus (figs. 50, 51). This condition will be discussed in greater detail when recording the results obtained with Regaud's technique. The plastids were not distinguishable with the Champy-Kull method after the nucleus began to coil.

#### *4. Results with Regaud's fixative and Heidenhain's haematoxylin stain*

This method fixes and stains both mitochondria and plastids. Mitochondria fixed by this technique are vastly different in appearance from those fixed with Champy-Kull's. Fixed with Regaud's fluid and stained with Altmann's stain they appear as short rods and as when fixed with Champy-Kull are far more numerous in the central cell than in the wall cells. The plastids, which are about equal in length to the mitochondria, are distinguishable from them by their heavily staining outer portion

and lightly-staining interior. The rod-like type of mitochondrion is present up to the formation of the spermatid. In the spermatid the mitochondria appear either as short rods (fig. 54) or as longer rods forming a more or less broken chain (fig. 58). At this stage the plastids also are still distinguishable (fig. 56). As the nucleus begins to coil, the rod-like mitochondria are found along its edges and scattered over its surface (figs. 59, 60). As the nucleus coils more and more the mitochondria become arranged in two rows, like two strings of beads, one along each edge of the nucleus on the inner surface of the coil and then extend to form a single row along that portion of the blepharoplast which protrudes beyond the nucleus (figs. 61, 62, 63).

#### DISCUSSION

##### *Morphological study of Polypodium polypodioides*

In studying the development of the prothallus of *Polypodium polypodioides* several problems of interest arose. It was found that antheridia and archegonia are produced in almost equal abundance on the upper as well as the lower surface of their respective prothallia. Reproductive organs appear first on the lower surface but soon form on the upper surface also. Another point of interest was the fact that archegonial plants very commonly formed proliferations from the margins and cushion of the prothallia. Mottier (23) has described these from cultures which he kept growing for many years. In *Polypodium polypodioides* they occurred on prothallia which were about six months old and which had apparently been exposed to no adverse conditions.

In the development of the archegonium it was found that sometimes a wall is formed between the two nuclei of the neck canal cell. This was noticed by Campbell (10) also in *Osmunda*. This condition in both *Osmunda* and *Polypodium polypodioides* is a rare occurrence.

The factor which prevents more than one sperm from entering the egg is as yet undetermined. Shaw (29) thinks that it is accomplished by the collapse of the egg after the sperm has entered. Mottier (21), on the other hand, thinks this collapsed condition is due to fixation. Strasburger (32) said that a heavy wall formed around the egg immediately after fertilization, and that this prevented additional sperms from entering. It was observed in *Polypodium polypodioides* that a few eggs which contained spermatozooids were collapsed. Many other eggs containing spermatozooids, in apparently the same stage, were turgid. Two conclusions might be drawn from this. Either the collapsed state of the recently fertilized egg is due to fixation or it is a normal process. If



it is normal the rounded fertilized eggs are older than the collapsed ones and have passed through that phase and later have regained their turgor. No unusually heavy wall was ever found around these eggs. Thus more evidence was obtained for Shaw's view than for the view held by Strasburger.

The development of the embryo of *Polypodium polypodioides* is much like that of other Polypodiaceae. Polyembryony, probably due to cultural conditions, was often found. As many as eight young embryos were sometimes found on a single plant and as many as three embryos on one plant might have one cauline leaf developed. This condition has been noted by Mottier (22) in certain Polypodiaceae and Osmundaceae.

#### CYTOLOGICAL STUDY

The nucleus and the blepharoplasts, as stated above in the review of the literature, are the objects which have received most attention in recent studies of spermatogenesis. The investigations of Belajeff (5, 6) and Shaw (30) on spermatogenesis in *Marsilea* and of Sharp (27) in *Equisetum* led them to conclude that the blepharoplasts in plant spermatozooids are morphologically the same as the centrosomes. They have also shown that the cilia arise from the blepharoplast. The work of Yamanouchi (37) on spermatogenesis in *Nephrodium* shows that the blepharoplast in that fern is lacking many of the characters of a centrosome. It does not divide to form daughter centrosomes, it only occasionally occupies the poles of the spindle and does not have astral radiations formed about it.

The blepharoplasts in *Polypodium polypodioides* first appear at the resting stage prior to the last division in the antheridium (fig. 24) as darkly-staining bodies, one on either side of the nucleus. During the last division they are found to occupy the poles of the spindle but no astral radiations are seen around them (fig. 25). When this division is completed and the daughter cells are formed there is found in each spermatid one large blepharoplast, situated in the cytoplasm at some distance from the nucleus (fig. 26). The chromatin of the spermatid nucleus at this time forms a coarse reticulum. The blepharoplast next approaches the nuclear membrane and finally comes in contact with it (fig. 27). After it has become applied to the nuclear membrane the blepharoplast begins to elongate. In the early stages of its elongation one end of the blepharoplast may be in contact with the nucleus while the other end extends out into the cytoplasm (fig. 28). As it elongates further, however, the blepharoplast extends half way around the still

globular spermatid nucleus. In preparations which show the coarse chromatin reticulum no nucleoli are seen. Other fixatives, however, brought out the fact that there are present in the spermatid one or two large nucleoli which are conspicuous until the nucleus begins to coil. After the blepharoplast has reached half way around the spermatid nucleus the chromatin begins to condense. The nucleus begins to flatten on the side opposite the blepharoplast and finally becomes concave (fig. 30). The chromatin continues to condense until it forms a solid, strap-like coiled body. The blepharoplast is applied to about one-third of the upper portion of the nucleus and extends one coil beyond the nucleus (fig. 31). This development of the blepharoplast and nucleus resembles that found in *Nephrodium* by Yamanouchi (37). Yamanouchi, however, does not find the blepharoplasts constantly located at the spindle poles during the last division, while in *Polypodium polypodioides* this does seem always to be true. Thus it appears that the blepharoplasts in this plant retain one centrosome-like character. The blepharoplast in this fern, as was also true in *Nephrodium*, is always a solid body. No segmentation occurs such as was found by Sharp (28) in *Marsilea* and *Equisetum* (27). The connections of the cilia with the blepharoplast were not seen nor could the writer find any division of the blepharoplast into a marginal hem and a cilium-bearing band such as Miss Dracinschi (12) describes.

*The nucleolus.* The changes in the nucleolus during spermatogenesis have not been discussed very extensively in most earlier studies of this stage. This is due to the fact that nucleoli are usually not very conspicuous in material fixed primarily for the study of chromatin. Yamanouchi (37) found either one or two nucleoli present in the spermatid of *Nephrodium*. And Sharp (27) in *Equisetum* figures from one to three nucleoli in the spermatogenous cells before their last division and but one nucleolus in the spermatid.

In *Polypodium polypodioides* the nucleoli stained very clearly with Heidenhain's haematoxylin after fixation with neutral formalin followed by Bouin's mixture. The central cell of the antheridium may contain one or two large, irregular nucleoli (fig. 33). In subsequent stages the nucleoli are smaller and more rounded. All nuclei in cells ranging from those in the two-celled antheridia to those of the spermatid contain either one nucleolus, a nucleolus in the process of constriction, or two nucleoli. For example, one four-celled antheridium was found in which three cells contained two nucleoli and one cell contained a nucleolus in the process of constriction. One eight-celled antheridium

was found with five nuclei containing two nucleoli and three nuclei containing one nucleolus each. Where there were two nucleoli in a nucleus these nucleoli were much smaller than that of a nucleus which had but one nucleolus. These facts give clear evidence that the nucleolar material in the antheridium of *Polypodium polypodioides* divides prior to nuclear division. The relation of the nucleolus to the chromatin reticulum has not thus far been studied since the neutral formalin rendered the chromatin unstainable. No trace of the nucleolus during mitosis was found.

*Development of the plastids.* In considering the development of the plastids in ferns it was thought advisable to review that phase of spermatogenesis in some other plants.

Gavaudan and Cazalis (15) studied the plastids in the spermatogenesis of the Characeae and found that the plastids containing carotin and starch stain distinctly up to the time that the spermatid nucleus begins to coil. At the time of the formation of the spermatozoid the plastids form a granular band on its posterior surface. A granular band was also seen in *Chara* by Muhldorf (25) who, however, found it to be composed only of starch.

Motte (20), working on spermatogenesis in the mosses, found a chondriome made up of long, narrow plastids and smaller mitochondria in the early stages of the antheridium. The plastids lose their starch and fragment to form, with the existing mitochondria, a granular mass which condenses to form a solid limosphere. In *Polytrichum*, Weier (36) found that one plastid remained in the androcyte, became reticulate and finally formed the limosphere. Then the limosphere, by division, formed the limosphere remnant and the apical body. The former contains starch and is found at the base of the spermatozoid; the latter is applied to the blepharoplast.

This work on the development of the plastids during spermatogenesis in the Characeae and in the mosses serves to emphasize several points. First, the behavior of the plastids during spermatogenesis varies in different groups. Second, the plastids in these groups have a complicated but regular developmental history during this process. Third, the plastids furnish the mature spermatozoid with the starch which is necessary to its functioning.

Yamanouchi (37), in his work on *Nephrodium*, did not discuss the early development of the antheridium. He does state, however, that the cytoplasm of the spermatid mother cell does not generally contain plastids. This was due, probably, to the fact that he used Flemming's

fixative which does not satisfactorily fix plastids except when they are mature.

Sharp (27), in his study of *Equisetum*, found plastids in various stages of disorganization up to the 8- or 16-celled stage and even in the spermatid. His interest at this time was, however, centered in the blepharoplast, hence in order to see these clearly he selected cells that contained the fewest plastids. The present work on *Polypodium polypodioides* does not indicate that the plastids disorganize but rather that they are present in all stages of spermatogenesis. The presence of these plastids could always be determined by their characteristic appearance during the early stages or in later stages by their contained starch.

Emberger (13) used Regaud's fixative in his study of spermatogenesis in ferns. He found that the wall cells decrease in size from losing their starch. The plastids of the central cell of the archegonium decrease in size until they are but slightly larger than the mitochondria. They can, however, be distinguished from the mitochondria by this slight excess of bulk. After the last division they form very small granules, practically indistinguishable from the mitochondria. A part of the granules migrate to the protoplasmic vesicle in the coil of the spermatozoid. He concludes that these bodies are the plastids since earlier workers, for example, Buller (8), found starch present in these vesicles. Recently, Muhldorf (24) also found starch in the protoplasmic vesicles of fern spermatozoids.

The plastids in the prothallia of *Polypodium polypodioides* are of the type described by Zirkle (39) for the higher plants. They are hollow, flattened spheroids. These plastids cover completely the exposed surfaces of the prothallial cells. They are composed of an outer chromatic stroma and an internal vacuole in which starch is stored. The plastids stain pink with Altmann's stain used after fixation with Champy-Kull's mixture. With this fixative and stain their development can be traced through all stages from the prothallial cells to the spermatid.

When the initial cells of the antheridium are cut off from the prothallial cells the plastids, which are found in the initials, are slightly smaller than those of the vegetative cells. The two divisions which follow in the initial cell divide that cell into an upper and lower wall cell and a central cell which is the primary spermatogenous cell (fig. 43). In the two wall cells the plastids are approximately of the same size as those of the antheridium initial. The plastids in the central cell on the other hand have decreased in size since they fail to grow after each division. They still possess, however, their characteristic structure, an outer

chromatic layer and an internal vacuole. Although the plastids vary in size, shape, and number in individual cells they are essentially alike in all cells of the spermatogenous tissue from the central cell to the formation of the spermatid (figs. 43-45).

After the spermatids are formed the plastids are no longer distinguishable in material fixed with Champy-Kull's fixative and stained with Altmann's stain. It was thus necessary to devise some method by which the presence of plastids in the spermatids could be detected. It was found after many unsuccessful attempts that material fixed in Regaud's mixture and stained by Gram's iodine method showed the presence of starch in the spermatid and in the spermatozoid. The starch was not noticeable in the younger stages of the antheridium with this method. It was then assumed that the starch had increased greatly in amount in the spermatid. These large starch grains in the plastids stretched the walls to such an extent that they could not be distinguished even after staining. Starch was also found in the spermatozoid when material fixed in Merkle's fixative was stained with Flemming's triple stain (fig. 52). With this method the starch in both the spermatozoid and the prothallial cells stained purple. The starch present in the spermatozoid is contained in the protoplasmic vesicle at the base of the spermatozoid.

From this work on the behavior of the plastids during spermatogenesis in *Polypodium polypodioides* it was found that the plastids play a definite rôle during this process. They can be identified in all cells before spermatid formation by size, structure, and staining reaction. After the spermatids are formed they can be identified by the starch which they contain.

*The chondriome.* There has been very little mention of the chondriome in connection with the discussion of spermatogenesis in plants, although it has long been known to be an important element in animal spermatogenesis.

In the mosses Motte (20) found that the mitochondria, and the fragmented plastids condense to form the limosphere. Weier (36) did not find any evidence of the chondriome in *Polytrichum*. He thought this might be due to some uncontrolled factor in the technique employed.

The only discussion of the behavior of the chondriome as such, during spermatogenesis in ferns, is that of Emberger (13). He finds that a few mitochondria are present in the antheridial initial and a larger number are present in the spermatogenous cells. When the nucleus of the spermatid begins to coil these granules are visible in the cytoplasm

surrounding it. In the mature coiled spermatozoid they are seen as two rows of granules along the inner surface of the coil. The mitochondria found in *Polypodium polypodioides* have essentially the same development and resulting position as that described by Emberger (13). There are points of variation which will be discussed later but these do not seriously interfere with the interpretation.

Two other workers, Sharp (27) and Miss Dracinschi (12), have described similar bodies in the mature spermatozoid. Sharp found a row of dark, rounded, bodies ranging, in *Equisetum*, along the inner surface of the coiled spermatozoid. He has mentioned them only to state that their nature and origin were not determined. Bodies of a similar appearance were also seen in the spermatozoid of the Eufilicinae and one of the Hydropteridinae, *Pilularia*, by Miss Dracinschi. She also could not explain their nature. The mitochondria in *Polypodium polypodioides* appear to be very similar to those described by Emberger (13) and those figured by Dracinschi (12) and by Sharp (27).

The mitochondria in *Polypodium polypodioides*, as has been stated above, differ somewhat from those of *Adiantum* described by Emberger (13). This difference is a matter of inconstancy in their shape, and in their size and arrangement at certain stages.

With Champy-Kull's fixative and Altmann's stain a few mitochondria are found to be present in the prothallial cells. They are short rods or granules which stain violet. They are found sometimes enveloping the plastids and sometimes in the cytoplasm between the plastids. During the division of the prothallial cell which forms the antheridium initial the mitochondria are found grouped about the spindle poles (fig. 42). In the initial cell of the antheridium the granules are more numerous than in the prothallial cells. The mitochondria are still more numerous in the wall cells of the antheridium than in the initial cells. However, they are fewer in number than in the central or primary spermatogenous cell. In this central cell there are exceedingly numerous purple granules, much smaller than the plastids, which are also present at that time (fig. 43). The mitochondria in all cells up to the formation of the spermatid have practically the same appearance as those of the central cell. The mitochondria are grouped about the poles of the spindle during the mitotic divisions of the spermatogenous tissue as was also true in the divisions which formed the antheridium initial.

When the spermatids are formed mitochondria are still abundant and scattered throughout the cytoplasm of the cell. The mitochondrial

granules begin to migrate toward the nucleus from the outer portions of the cytoplasm when the nucleus of the spermatid begins its metamorphosis. They can be observed scattered over its outer surface when the nucleus is partially coiled; however, they are usually more numerous along the margins of the coil (fig. 49). By the time the chromatin of the nucleus has condensed to form a nearly solid body the mitochondria are found as a double row of granules along the inner surface (figs. 50, 51).

Regaud's fixative gave two quite different results. In some material the mitochondria appeared as short rods in the prothallial cells, all the antheridial cells, and spermatids (fig. 54). The mitochondria grouped themselves about the metamorphosing nucleus and finally migrated to the under surface where they formed the two rows of granules. In other prothallia fixed with Regaud's fixative the mitochondria were seen to form a broken network in the antheridium initial instead of forming separate rods (fig. 53). This network was observed also in the different stages of the antheridium up to the last division of the spermatogenous tissue. In the spermatid this network was especially noticeable and while the nucleus is central these mitochondria seem to surround the nucleus on all sides (figs. 57, 58).

After the nucleus has condensed as in the preceding cases, the mitochondria form the double row of granules around the inner surface of the spermatozoid (figs. 61-63). Between the rod-like mitochondria and the mitochondria which formed the broken network there were many intermediate stages. It was thus concluded that there was either some slight variation in technique during different fixations or that the physiological condition of the prothallia varied. Either, it is believed, could account for a difference in the morphological character of the mitochondria.

The differences in the mitochondria as shown by Champy-Kull's and by Regaud's fixatives must be due to technique. The fact that in both cases the granules form a double row along the inner surface of the spermatozoid is proof of the fact that these are identical structures.

#### SUMMARY

*Morphological.* Antheridia appear on prothallia of *Polypodium polypodioides* when the plants are about two months old. Archegonia appear about two weeks later on other plants of the same sowing which are approximately three times as large as the male plants mentioned. Both types of organs occur on both upper and lower surfaces of the

prothallia. Antheridia and archegonia occurred on the same plants simultaneously only where antheridia arose on proliferations of plants that had already borne archegonia.

The development of the antheridium and archegonium corresponds to that in many other Polypodiaceae. The opening of the antheridium is by the extrusion of the cover cell with wall intact or as a granular mass. In the archegonium, a wall is occasionally formed separating the two nuclei of the neck canal cells.

The ripe antheridia and archegonia open in a few minutes after the prothallia are watered. Many spermatozoids enter the open neck of the archegonium, but only one penetrates the egg. The egg is turgid before the penetration of the sperm. Many of the eggs observed immediately after fertilization were in a collapsed condition. No especially heavy wall was formed around the recently fertilized eggs.

*Cytological.* The blepharoplasts arise in the resting stage of the spermatogones before the last nuclear division in the antheridium. They occupy the poles of the spindle during the subsequent mitosis. When the spermatids are formed one blepharoplast is found in each cell and is situated at some distance from the nucleus. The blepharoplast moves toward the coarsely reticulate nucleus and becomes applied to the nuclear membrane. Here it elongates until it has reached one-half way around the still globular spermatid nucleus. At this time the nucleus begins to condense and forms a strap-like coiled body. The blepharoplast, in a mature spermatozoid which is still inside the antheridium, has stretched to form about four-fifths of a complete coil and the nucleus forms about one and a half coils. The blepharoplast extends beyond the nucleus approximately half of a coil.

The structure and development of the nucleoli was traced, using material fixed in neutral formalin followed by Bouin's fluid. One or two large irregular nucleoli are found in the central cell of the antheridium. One or two nucleoli are also almost constantly found during the subsequent generations of the spermatogenous tissue. Oftentimes a constricted nucleolus was observed. These latter were seen even after the spermatid was formed and the blepharoplasts had begun their elongation. Where there are two nucleoli in a nucleus the nucleoli are much smaller than the single nucleolus which is sometimes found in each nucleus. The presence of sometimes one nucleolus, of two nucleoli, or in other cases of a constricting nucleolus, and the relative sizes of the nucleoli, all indicate that the nucleolus divides prior to each nuclear division.



The plastids were identified by their morphological characteristics in the prothallial cells, the antheridium initial, the wall cells, and in all spermatogenous cells until the spermatids were formed. After the formation of the spermatids the plastids were identified by their contained starch. This starch is abundant in the spermatids and mature spermatozoids. No starch was found in younger stages. The plastids of the spermatids were stretched to such an extent by enclosed starch that their boundaries could not be seen, even after staining.

A few rod-like or granular mitochondria are found in each prothallial cell. They are either grouped around the plastids or are scattered in the cytoplasm. In the division prior to the formation of the antheridium initial they are grouped about the poles of the spindle. The mitochondria are found in the antheridium initial and the antheridial walls, but are not as numerous there as in the spermatogenous tissue.

The mitochondria appear as short rods or granules with Champy-Kull's fixative and Altmann's stain. With Regaud's fixative they appear as long rods in some fixations and in other fixations have the appearance of a broken network. The mitochondria are very numerous in all stages of the spermatogenous tissue when both Champy-Kull's and Regaud's fixatives are used.

Soon after the nucleus begins to condense the mitochondria migrate from the peripheral cytoplasm toward the nucleus. They form a sheath around the partially condensed nucleus. The granules continue their migration as the spermatozoid matures. And in the fully developed spermatozoid they are found as two beaded rows along the inner surface of the coiled nucleus and extending as one row along that portion of the blepharoplast which protrudes beyond the nucleus.

It is a pleasure for the author at this time to acknowledge her indebtedness to Professor Duncan S. Johnson who suggested this problem and under whose direction this work was carried on. She would like also to thank Professor Conway Zirkle of Philadelphia for suggesting certain fixatives used and Professor Ralph E. Cleland of Baltimore for very helpful advice.

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### DESCRIPTION OF PLATES

Figures 1-3 are photographs of living prothallia. Figures 4-63 are from paraffin sections of fixed prothallia. Figures 22-32 were fixed with Flemming's fixative and stained with Heidenhain's haematoxylin. Figures 33-40 were fixed with neutral formalin and Bouin's fluid and stained with Heidenhain's haematoxylin. Walls of cells and chromatin reticulum not stained. Figures 42-51, fixative, Champy-Kull's; stain, Altmann's. Figure 52, fixative, Merkel's; stain, Flemming's triple, walls in spermatogenous tissue not stained. Figures 53-63, fixative, Regaud's; stain, Heidenhain's haematoxylin.

#### General Explanation of Plates

All prothallia were cut parallel to the axis.

All figures were drawn with the aid of the Abbé camera lucida (Zeiss).

Magnifications (of figures as printed)

Figures 4-10 =  $\times 450$

Figures 11-13, 22 and 23 =  $\times 650$ .

Figures 14-19 =  $\times 270$ .

Figure 20 =  $\times 170$ .

Figure 21 =  $\times 90$ .

Figures 24-63 =  $\times 2000$

#### PLATE 23

Fig. 1. Antheridial plant in which the notch is formed.

Fig. 2. Male prothallium: antheridia cover whole surface except the three anterior rows of cells; some spermatozoids discharged.

Fig. 3. Culture of prothallia of *Polypodium polypodioides* including larger archegonial plants and smaller antheridial ones.

#### PLATE 24

Fig. 4. Vertical section of antheridium initial showing first mitosis.

Fig. 5. Three-celled archegonium.

Fig. 6. Young archegonium with two neck cells.

Fig. 7. First mitosis of central cell of archegonium.

Fig. 8. Ventral cell of archegonium in mitosis.

Fig. 9. Mature archegonium showing wall separating the neck canal cells; egg indented.

Fig. 10. Mature archegonium showing shallow egg cell: ventral cell; and two neck canal cell nuclei.

Fig. 11. Spermatozoid entering egg.

Fig. 12. Spermatozoid within the egg nucleus.

Fig. 13. Spermatozoid nucleus within egg nucleus, chromatin of former becoming reticulate.

Fig. 14. One-celled embryo.

Fig. 15. Two-celled embryo: wall slightly oblique to neck of archegonium.

Fig. 16. Four-celled embryo.

Fig. 17. Young embryo.

Fig. 18. Young embryo.

Fig. 19. Embryo showing primary organs differentiated; octant walls still distinct.

Fig. 20. Embryo slightly older than in fig. 19; octant wall distinct, but irregular.

Fig. 21. Older embryo: cotyledon initial; region of stem-growing point; foot; region of root-growing point.

#### PLATE 25

Fig. 22. Antheridium showing upper and lower wall cells and central cell.

Fig. 23. Antheridium with upper and lower wall cells, central cell and cap cell.

Fig. 24. Spermatid mother cell: two blepharoplasts.

Fig. 25. Spermatid mother cell in prophase: two blepharoplasts.

Fig. 26. Spermatid: blepharoplast in cytoplasm at some distance from nucleus.

Fig. 27. Spermatid: blepharoplast applied to nuclear membrane.

Fig. 28. Spermatid: blepharoplast beginning to elongate.

Fig. 29. Spermatid: nucleus beginning to condense; blepharoplast more than half around nucleus.

Fig. 30. Crescent shaped spermatid nucleus.

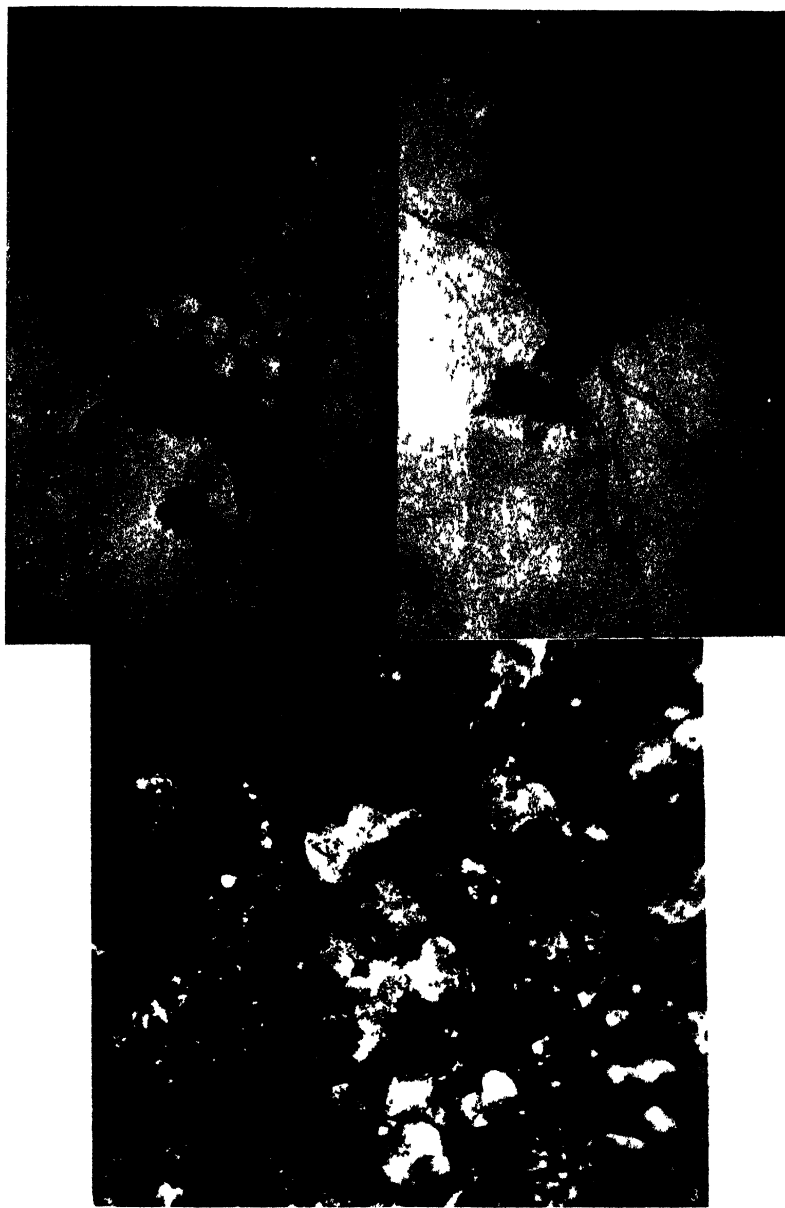
Fig. 31. Coiled spermatozoid: nucleus condensing; blepharoplast extending beyond nucleus.

- Fig. 32. Spermatozoid found in neck of archegonium: 2.5 coils; cilia distinct.  
Fig. 33. One-celled antheridium: central cell with two large irregular nucleoli.  
Fig. 34. Two-celled antheridium: one nucleus with two nucleoli; one with constricting nucleolus.  
Fig. 35. Four-celled antheridium: three nucleoli in section; two nuclei with one nucleolus each; one nucleus with constricting nucleolus.  
Fig. 36. Eight-celled antheridium: four cells in section; three nuclei with two nucleoli; one nucleus with constricting nucleolus.  
Fig. 37. Spermatid: blepharoplast near nucleus; two nucleoli.  
Fig. 38. Spermatid: blepharoplast applied to nuclear membrane, one nucleolus.  
Fig. 39. Spermatid: blepharoplast applied to nuclear membrane; two nucleoli.  
Fig. 40. Spermatid: blepharoplast elongated; one nucleolus.  
Fig. 41. Spermatid: blepharoplast elongated; two nucleoli.

## PLATE 26

- Fig. 42. Prothallial cell dividing to form antheridium initial: large plastids; mitochondria near poles of spindle.  
Fig. 43. Antheridium showing central cell and two wall cells; plastids in wall cells large; plastids and mitochondria in the central cell.  
Fig. 44. Two-celled antheridium: plastids in wall cells; plastids and mitochondria in spermatogenous tissue.  
Fig. 45. Four-celled antheridium: plastids and mitochondria.  
Fig. 46. Spermatid mother cell at metaphase: blepharoplasts at poles of spindle.  
Fig. 47. Spermatid—blepharoplast applied to nuclear membrane; mitochondria in cytoplasm.  
Fig. 48. Spermatid: nucleus beginning to flatten; blepharoplast half way around nucleus; mitochondria in cytoplasm.  
Fig. 49. Spermatid: part of coiling nucleus; mitochondria on surface of nucleus and in nearby regions of cytoplasm.  
Fig. 50. Mature spermatozoid: blepharoplast applied to anterior portion of nucleus and extending .5 of a coil beyond it; mitochondrial granules along inner surface of blepharoplast and nucleus.  
Fig. 51. Mature spermatozoid: blepharoplast applied to anterior portion of nucleus and extending .5 of a coil beyond it; mitochondria as two rows along inner surface of nucleus and one row along blepharoplast.  
Fig. 52. Mature spermatozoid with starch in protoplasmic vesicle.  
Fig. 53. Antheridium initial: mitochondria in broken network.  
Fig. 54. Spermatid: mitochondria as short rods.  
Fig. 55. Spermatid: mitochondria as short and long rods.  
Fig. 56. Spermatid: mitochondria as short rods; plastids.  
Fig. 57. Spermatid: mitochondria as short and long rods.  
Fig. 58. Spermatid: mitochondria forming broken network.  
Fig. 59. Spermatid nucleus beginning to coil; mitochondria surrounding nucleus.  
Fig. 60. Spermatid: nucleus coiling; mitochondria around nucleus.  
Fig. 61. Mature spermatozoid: two rows of mitochondria along inner surface of nucleus; one row extending beyond it.  
Fig. 62. Mature spermatozoid showing two rows of mitochondria.  
Fig. 63. Mature spermatozoid showing two rows of mitochondria.

PLATE 23





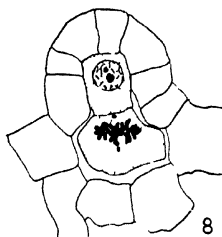
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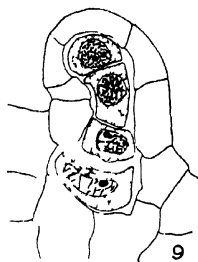
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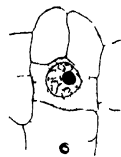
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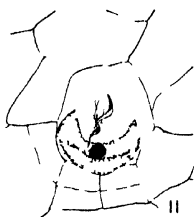
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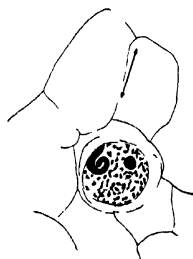
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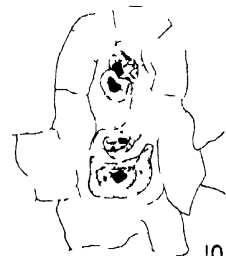
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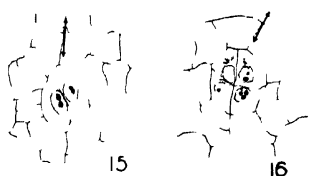
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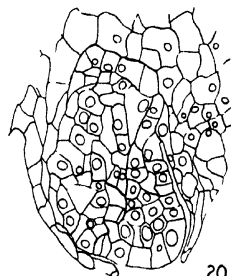
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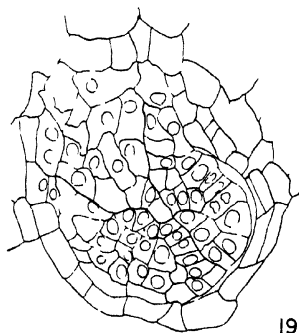
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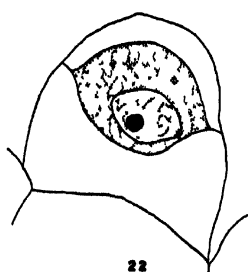


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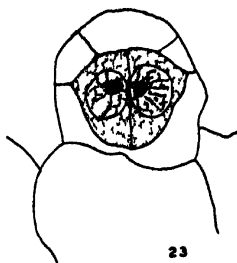




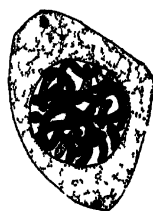
# PLATE 25



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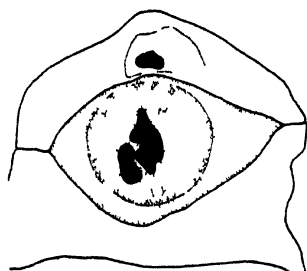
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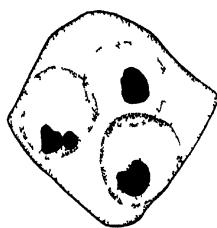
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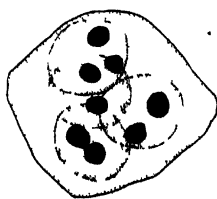
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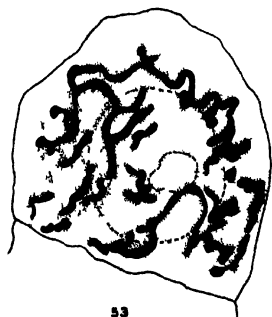
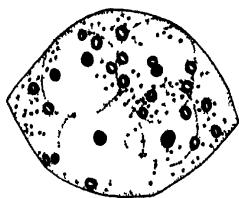
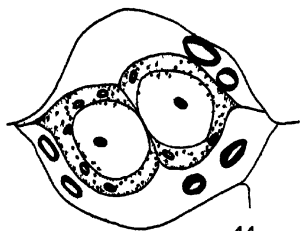
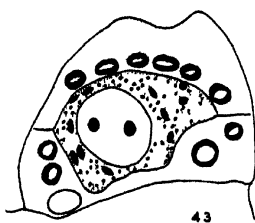
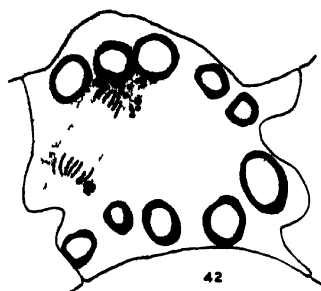
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# PLATE 26



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